OXFORD CANCER

DPhil in Cancer Science

University of Oxford

2024 Intake Project Book

CANCER RESEARCH UK OXFORD CENTRE

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UNIVERSITY OF OXFORD

BLACK ACADEMIC FUTURES

DPhil in Cancer Science
2024 Intake Project Book

Introduction

This handbook provides an overview for prospective students looking to study for a DPhil in Cancer Science starting in 2024 at Oxford University. The Programme provides research based doctoral training for cancer researchers from clinical, biological, engineering, mathematics, and statistics background. Students will receive a world-leading research training experience that integrates an education initiative spanning cancer patient care, tumour biology and research impact; on- and post-programme mentorship; and a specialised, fundamental, subject-specific training tailored to individual research needs. Students participating in the scheme will be offered:

- a choice of interdisciplinary cutting-edge cancer research projects.
- the ability to gain a working in-depth knowledge of the fundamentals of cancer biology and cancer patient care through advanced level seminars.
- a world-renowned research environment that encourages the student's originality and creativity in their research.
- opportunities to develop skills in making and testing hypotheses, in developing new theories, and in planning and conducting experiments.
- an environment in which to develop skills in written work, oral presentation and publishing the results of their research in high-profile scientific journals, through constructive feedback of written work and oral presentations.

At the end of their DPhil course, students should:

- have a thorough knowledge of the basic principles of cancer research including the relevant literature and a comprehensive understanding of scientific methods and techniques applicable to their research.
- be able to demonstrate originality in the application of knowledge, together with a practical understanding of how research and enquiry are used to create and interpret knowledge in their field.
- have developed the ability to critically evaluate current research and research techniques and methodologies.
- be able to act autonomously in the planning and implementation of research.
- have the grounding for becoming an influential cancer researcher of the future.

Selection Criteria & Eligibility

There are four tracks in the programme as described below, meaning that non-clinicians, undergraduate medical students and post-graduate medical trainees are all eligible to apply for the fully funded (at home rate) studentships.

Application Track 1 – Clinical Trainees. Qualified doctors at all stages of training from the foundation training to higher specialist training.

Application Track 2 – Medical Undergraduates. Medical students who are currently undertaking a primary medical qualification (MBBS, MBChB or equivalent). At entry, we will be looking for evidence of completion of at least the first two years of a primary medical qualification and achievement at the level of an upper-second or first-class honours degrees (or iBSc).

Application Track 3 – Non-Clinical/Fundamental Scientist. Science graduates that hold (or be predicted to achieve) the equivalent of a first-class or strong upper second-class undergraduate degree with honours in biological, medical, or chemical science, as appropriate for the projects offered.

Application Track 4 – Non-Clinical/Fundamental Scientist. Science graduates that hold (or be predicted to achieve) the equivalent of a first-class or strong upper second-class undergraduate degree with honours in engineering, mathematical/data, ***or*** physical science, as appropriate for the projects offered.

All applicants will be judged on the following:

- commitment and passion to a career in cancer research
- evidence of motivation for and understanding of the proposed area of study
- commitment to the subject, beyond the requirements of the degree course
- preliminary knowledge of relevant research techniques
- capacity for sustained and intense work
- reasoning ability and academic curiosity.

Funding

All offered places are fully funded at the home rate. This includes salary/stipend, University/College fees, and a research consumables budget of ~£13k p.a.
Salary and stipend provisions are summarised below:

- **Application Track 1:** 3 years of salary at Grade E63 or E64 Clinical Researcher rate.
- **Application Track 2:** 3 years of stipend at the flat rate of £21,000 per annum.
- **Application Tracks 3 & 4:** 4 years of stipend at the flat rate of £21,000 per annum.

International applicants are eligible; however, funding is limited to the Home level for this programme and therefore international applicants would need to either source further funding or support themselves financially for the remaining fees.

Notable Scholarships

Black Academic Futures Scholarships
These awards offer UK Black and Mixed-Black students scholarship funding to pursue graduate study at Oxford, alongside a programme of on course mentoring and support. The Medical Sciences Division has guaranteed places across its DPhil courses (including the DPhil in Cancer Science). For more information, visit the Black Academic Futures website.
To receive a Black Academic Futures Scholarship, submit your application to the DPhil in Cancer Science Programme by the December deadline. All those that include eligible ethnicity will automatically be considered. You do not need to submit any additional documents and there is no separate scholarship application form for these awards.

How to Apply

A detailed summary on how to apply can be found here. In brief, prospective students apply with a prioritised list of three projects selected from this booklet by **Friday 1st December 2023.** Shortlisted students will be invited to interview in January. If successful, students will be allocated a project on the basis of their ranking during the review process. It is strongly suggested that students contact supervisors of projects they are interested in applying for prior to application.

Projects

Projects are listed below in the following structure "Title Eligible Application Tracks – Primary Supervisor Page number."

Clicking on the project title below will take you to the project page.

Project Number **Page Number**

1. Comprehensive Proteomics Study of Oncogenic JAK2 as a Basis for Improved Therapies Against Myeloproliferative Neoplasms [1,2,3,4] – Dr Adán Pinto-Fernández

Primary Supervisor: Dr. Adán Pinto-Fernández
Additional Supervisors: Prof. Benedik Kessler
Eligibility: All tracks are eligible to apply for this project.

Abstract of the project
Myeloproliferative neoplasms (MPN) are progressive blood cancers where the bone marrow overproduces mature white blood cells. Of interest, an activating mutation in a cellular protein called JAK2 is found in most MPN cases. Ruxolitinib (Jakafi) is a highly potent and selective JAK2 inhibitor approved for MPN in 2011 and has demonstrated major benefits to patients. However, most patients with mutated JAK2 do not respond to this drug and, therefore, the identification of more efficient therapies remains critical. (More info in references 1 and 2, at the end of the proposal). This PhD project aims to understand why current therapies targeting JAK2 for the treatment of myeloproliferative neoplasms do not work in patients carrying the above-mentioned mutation. Understanding the functional consequences of this mutation will provide novel insights for overcoming resistance to therapy and eventually improve the survival and life quality of MPN patients. We performed a comprehensive mass spectrometry-based interactomic, proteomic, and phospho-proteomic analysis to identify proteins associated with activated JAK2 in five different blood cancer cell lines harbouring wildtype JAK2 (TF1 and F36P) and the mutation associated with MPN: JAK2 V617F (HEL, SET2, and UKE1). The cells were treated with Erythropoietin (EPO) at different time points (0, 5 min., 15 min., and 30 min.) in the presence/absence of two JAK2 inhibitors, 1824 (Ruxolitinib) or 532. Interactome data has been fully analysed and proteome and phospho-proteome data have been acquired and preliminarily analysed. ***The proposed research involves the validation of the interactome data, the analysis of the proteome and phospho-proteome data and the study of JAK2 roles in MPN resistance to immunotherapy***. Project overview in **Figure 1**.

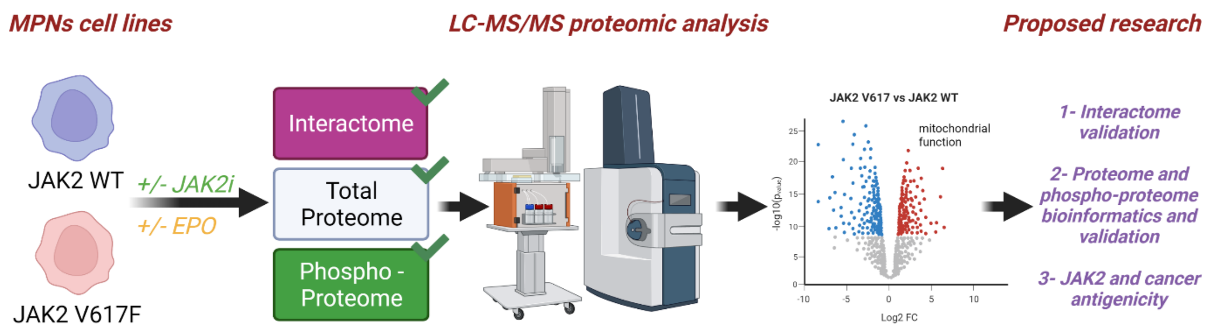

Figure 1: Discovery of JAK2 wildtype versus V617F mutant dependent molecular pathways. Proteomic workflow including interactome, phospho-proteome, and total proteome analysis of five different blood cancer cell lines. Proposed research includes the validation of the JAK2 interactome, the comprehensive bioinformatic analysis of the total proteome and phosphor-proteome data and the study of JAK2 in cancer immunogenicity.

Analysis of the JAK2 interactome unveiled the **association of wildtype JAK2 with mitochondrial components** upon activation with EPO. Notably, JAK2 inhibitors did prevent EPO-dependent binding of JAK2 WT to such mitochondrial components. Most importantly, this **regulation seemed to be lost in cells harbouring the activating V617F mutation (Figure 2)**, unveiling a potential resistance mechanism in the cells with the mutation.

Our results represent novel biology associated with JAK2 signalling and EPO activation cascades and could possibly provide a potential link to oxygen sensing and energy metabolism, affecting cellular proliferation and differentiation. Supporting these observations, previously published data described that interfering with mitochondrial functions induced cell death in MPN cells containing the activating mutation in JAK2. In summary, the appointed PhD student, with the guidance of experts in cancer research and applying advanced biomedicine and bioinformatics tools, will aim to validate and expand the above-described findings in order to better understand how mutated JAK2 works and to identify novel, more efficient, treatments for MPN.

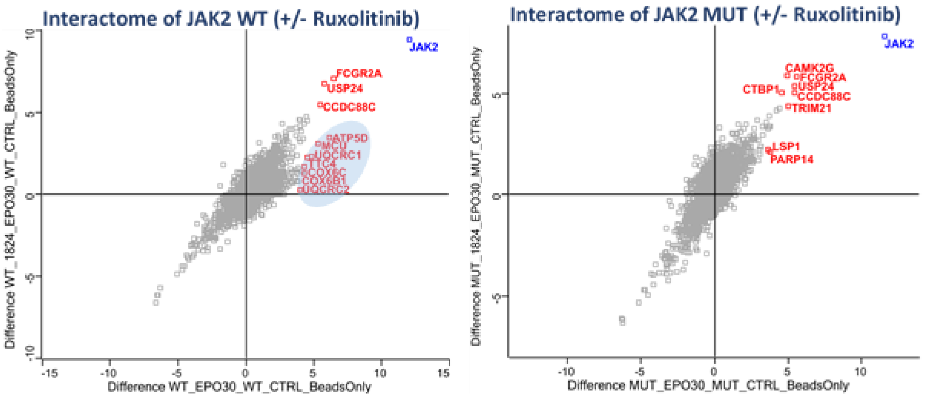

Figure 2: JAK2 inhibition prevents association of mitochondrial components to wildtype (circled in blue), but not to V617F JAK2. Comparative scatter plots showing the JAK2 interactomes in WT cells (left) and JAK2V617F cell lines (right). Conditions EPO 30 minutes treatment in the presence/absence of JAK inhibitor 1824 (Ruxolitinib) were plotted. The bait is labelled in blue and selected interactors in red.

Research objectives and proposed outcomes

- To study the co-localisation/interaction between JAK2 (mutant and WT), upon EPO stimulation (+/- JAK inhibitor and +/- JAK2 genetic inhibition), and the identified mitochondrial components by co-immunoprecipitation and confocal microscopy. These experiments aim to validate the proteomics data.
- To measure mitochondrial activity reflected by oxygen consumption rate (OCR) and extracellular acidification rates (ECAR) in the same conditions using a Seahorse Real-Time Cell Metabolic Analyser and ROS production using molecular probes in a live cell imaging system.
- Evaluate whether combined use of JAK2 inhibitors and ROS inducers such as LAAO, CCCP, Rotenone, Cisplatin, enhances MPN anti-tumour therapy.
- Comprehensive bioinformatic analysis of the remaining data (phospho-proteome and total proteome) and further validation.
- Study of JAK2-dependent tumour antigenicity in T cell activation/T cell killing assays (in collaboration with Prof. Tao Dong).

Translational Potential

With this research plan, we expect to uncover novel JAK2 biology that could explain resistance mechanisms to current therapy and allow the characterisation of additional targets for the treatment of MPN. With the first three objectives of the proposal, we want to validate our interactomics data suggesting that JAK2, upon activation, has important roles in mitochondrial function and cellular metabolism. For instance, it has been shown that mutated JAK2 induced the accumulation of reactive oxygen species (ROS) in patients with MPN, highlighting key roles for the JAK2 pathway in mitochondrial activity and intracellular ROS generation (reference 3). The authors of this study suggested that targeting ROS accumulation might prevent the development of JAK2V617F MPN. Further supporting this hypothesis, another study demonstrated that a ROS inducer exhibited cytotoxicity and induced apoptosis in JAK2V617F MPN cell lines in a ROS production-dependent manner (reference 4). Based on this, we will determine whether ROS inducers enhance the anti-cancer effects of JAK2 inhibition. Importantly, additional JAK2 biology will be explored using the remaining proteomic analysis, potentially providing additional insights into resistance to JAK inhibition. Finally, the interplay between JAK2 status/inhibition and its implications to resistance to current immunotherapy have been described (reference 5) and will be investigated using relevant T cell models.

Summarise the training opportunities.

The student will have opportunities to gain expertise in advanced bioinformatics, proteomics, metabolic studies (Seahorse and mass-spectrometry), cellular biology (immunofluorescence, tissue culture, live cell imaging, gene knockdown/knockout etc), immunology (T cell activation and T cell killing) and biochemistry (immunoprecipitation and immunoblotting).

Relevant References

1.Schieber M, Crispino JD, Stein B. Myelofibrosis in 2019: moving beyond JAK2 inhibition. Blood Cancer J. 2019;9(9):74.
2.Staerk J, Constantinescu SN. The JAK-STAT pathway and hematopoietic stem cells from the JAK2 V617F perspective. JAKSTAT. 2012;1(3):184-90.
3.Marty C, Lacout C, Droin N, Le Couedic JP, Ribrag V, Solary E, et al. A role for reactive oxygen species in JAK2 V617F myeloproliferative neoplasm progression. Leukemia. 2013;27(11):2187-95.
4.Machado-Neto JA, Traina F. Reactive oxygen species overload promotes apoptosis in JAK2V617F-positive cell lines. Rev Bras Hematol Hemoter. 2016;38(3):179-81.
5. Horn S, Leonardelli S, Sucker A. et al. Tumor CDKN2A-Associated JAK2 Loss and Susceptibility to Immunotherapy Resistance. J Natl Cancer Inst. 2018; 1;110(6):677-681

2. Targeting radio-ligand therapy to protonated CEACAM6 epitopes induced in acid-resistant cells under improved oxygen – release from biochemically rejuvenated transfusions [2,3,4] - Prof. Pawel Swietach

Primary Supervisor: Prof. Pawel Swietach
Additional Supervisors: Prof. Katherine Vallis and Prof. Simon Newstead
Eligibility: Track 2, 3 and 4 students are eligible to apply for this project.

Abstract of the project
All under-perfused tumours accumulate acidic end-products of metabolism. Successful cancer cells adapt to acidosis and eliminate competition from acid-sensitive neighbours by releasing more acid. The outcome of this process is acid-driven Darwinian evolution, which we propose to derail by specifically killing acid-resistant colorectal cancer (CRC) cells using radioligand therapy (RLT) that targets acid-induced epitopes in their protonated conformation. Our CRISPR/Cas9 screen determined that Complex I genes are essential for survival at low pH, consistent with a greater reliance on respiration when acidosis blocks glycolysis. In a panel of colorectal cancer cell lines, we described CEACAM6 as a surface-expressed marker of such acid-resistant phenotypes. We will target this epitope using synthetic peptides inspired by CEACAM-binding dr-adhesins produced by *E. coli*. To target the protonated confirmation, peptides will be generated at low pH. Validated peptides will be linked with radionuclides to selectively kill acid-resistant cells in spheroid and xenograft models. It is unclear whether tumour hypoxia is problematic for RLT efficacy in CRC patients who often become anaemic (e.g. rectal bleeding). Transfusions increase haematocrit but stored red blood cells (RBCs) have reduced O_2-release kinetics and may not improve tumour oxygenation. However, we demonstrated how O2-unloading can be improved by in-bag biochemical rejuvenation, which we propose to combine with RLT to improve efficacy in vivo. This will be tested in xenograft models established in mice receiving standard-storage or rejuvenated blood. The project capitalizes on recent advances in acid-driven oncogenic progression to develop a new line of therapy that exploits tumour-specific features.

Research objectives and proposed outcomes
Objective 1*: Verifying the target (months 0-9):* We have characterized the mechanisms of acid-adaptation and identified CEACAM6 as the surface-expressed marker of acid-resistant phenotypes. Moreover, acid-resistant lines induce further *CEACAM6* gene expression within 12h exposure to acidity. We will verify CEACAM6 as a marker of acid-resistant cells in spheroids and xenografts by correlating with its distribution for pHLIP, a peptide that integrates into membranes in acidic niches. Next, we will map the acid-induction signalling pathway. A candidate negative-regulator is the transcription factor CDX1, which we will test by immunofluorescence and promoter binding (ChIP-qPCR). We believe that these mechanisms may be general in cancer, e.g. our pilot data in PDAC, a cancer that develops from ductal epithelia that are basolaterally acidic. Thus, there is potential for protonated CEACAM6 to be a broad-spectrum cancer target, which we will test in a panel of cell lines. Our group has expertise in studying acid-evoked processes *in vitro* and in animal models. This part of the project is low-risk because it is supported by strong pilot data, and will build the student's confidence.
Objective 2: *Refining and testing the ligand (months 10-24; preliminary work prior to transfer viva):* We will develop ligands, suitable for development as theranostic agents, that bind preferentially to protonated CEACAM6. Candidate peptides are based on bacterial dr-adhesins that bind in a highly conformation-sensitive manner. Whilst antibodies to a related isoform, CEACAM5, have been raised by others, our aim is to seek ligands that selectively bind at low pH to minimize off-target actions in non-acidic tissues or on circulating CEACAM6 fragments. For this, we will adopt a strategy that we developed for generating synthetic nanobodies (sybody), involving phage display. Optimized sequences will be used to prepare ligands for conjugation with fluorophores for testing accuracy of targeting in CRC spheroids. Successful ligands will be conjugated with radionuclides via bifunctional metal chelators, focusing initially on Lutetium-177 for therapy and SPECT imaging, and Gallium-68 for PET imaging. Novel peptide- and sybody-based ligands will be evaluated *in vitro* in competitive binding and cytotoxicity assays, and their radiolabelling yields (RLY) determined. *In vivo* microPET/SPECT-CT imaging, biodistribution/PK and tumour/normal organ dosimetry studies will be performed in tumour-bearing mice. The cancer cell killing efficacy and selectivity of radiolabelled ligands will be tested initially in spheroid and then xenograft co-cultures established from ad-mixtures of acid-resistant and acid-sensitive CRC cell lines. Relevant readouts include histological measures of the balance between acid-resistant and acid-sensitive cells, overall tumour growth and survival. These experiments will enable dosage optimization for further testing in future experiments on other animal models of cancer, such as orthotopic or genetically-engineered mice. This part of the project will involve screening peptides for target binding and has an element of risk, which we mitigate by using bacterial proteins as a starting point.
Objective 3*: Radio-sensitisation to enhance RLT anti-cancer effects (months 25-36):* Many tumours are hypoxic, but the effect of reduced oxygenation on RLT efficacy is unclear. Based on the favourable effect of raising tissue O_2 availability on external

beam therapy (EBRT), it is plausible that RLT efficacy is sub-optimal in hypoxic microenvironments, which also harbour the acid-resistant targets. Oxygen availability can be raised by blocking the electron transport chain (e.g. atovaquone), but this may have off-target effects. We will test a novel approach that stems from our work on oxygen-handling by RBC. Our research has found that O_2-unloading from RBCs is slower than previously estimated. This arises from restricted gas diffusion across the 'crowded' RBC cytoplasm. The size of this diffusional barrier can cause O_2 supply to tissues to become diffusion-limited. In blood-units stored for transfusion, RBCs undergo spherical remodelling which lengthens intracellular pathlength and slows O_2 release. We showed that kinetically-slow RBCs resulted in poor O_2 extraction in perfused kidneys. Anaemic CRC patients are predisposed to hypoxia but transfusions with kinetically-compromised blood may not improve tissue oxygenation. We refined an in-bag biochemical rejuvenation process that accelerates O_2 release and postulate that transfusions with such bloods can improve tumour oxygenation, without affecting other parameters such as acidity. To test this intervention, we will wean mice on an iron-deficient diet which causes anaemia. Once haematocrit stabilizes, subcutaneous xenografts will be established and mice will be transfused with stored bloods randomized to receive rejuvenation. The efficacy of this intervention will be measured histologically by pimonidazole staining. Our findings may impact other cancer treatments, such as EBRT. This proof-of-principle part of the project may use any form of RLT, and does not necessary require objective 2 to be met

Translational Potential

Acid-driven disease progression is a well-established concept in cancer that is rooted in Darwinian evolution. The current strategy for interfering with acid-selection is to remove tumour acidosis (the selection pressure) by using buffers (e.g. oral bicarbonate) or by urase enzymes that produce buffer. Despite excellent preclinical results, acid-buffering is not without problems in humans, such as compliance to take large amounts of bicarbonate. We propose an alternative approach that attenuates acid-driven progression by eliminating the acid-adapted cells. By targeting the protonated conformation, we anticipate good selectively over non-cancer cells expressing similar proteins. Mathematical modelling predicts that eliminating the fittest cells should permanently swerve the disease trajectory from its most malignant form. Our second translational deliverable is the use of rejuvenated blood products to improve tumour oxygenation, which we predict may improve RLT and EBRT. Attempts have been made to raise oxygen availability by blocking mitochondria but this can be problematic systemically. Our method is less invasive and addresses a niche in the area of transfusion medicine, which is a major part of patient care. Indeed, anaemia is a common co-morbidity in cancer patients.

Training opportunities

Our host institution has state-of-the-art facilities, resources, and expertise to undertake the project. This includes cancer physiology, RLT, nuclear imaging, biophysics and nanobody production. We collaborate with clinical partners and the blood service, and our cross-disciplinary approach opens new lines of discovery science. We offer training across three Oxford departments: in acid-base physiology and oxygen transport, oncology and RLT, and peptide biochemistry. Our team has a track-record in CRC research, with unique access to ~100 CRC lines and corresponding spheroid and xenograft models. RLT is an emerging area of oncology, with much optimisation ahead and therefore with ample opportunities to make impact. The research project implements risk-mitigating steps to ensure that the work-packages are not entirely contingent on success in prior objectives. Overall, the student will have experienced a range of methods from synthetic protein chemistry to animal studies; from basic-research to translational deliverables. This project uniquely offers opportunities to collaborate with England's blood service, NHS Blood & Transplant, to improve transfusion products specifically for cancer patients. This area is novel because it stems from discoveries made by our labs in the last three years.

References

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3. Understand the developmental origin of Clear Cell Sarcoma using long-read single cell sequencing .[1,3,4] – Assoc Prof. Sarah Snelling

Primary Supervisor: Assoc Prof. Sarah Snelling
Additional Supervisors: Assoc Prof. Adam Cribbs
Eligibility: Track 1, 3 and 4 students are eligible to apply for this project.

Abstract
Clear Cell Sarcoma (CCS) of tendons, is a rare malignant soft tissue sarcoma, typically derived from neural crest cells. It usually presents in the distal lower extremities of young adults, frequently attached to tendons. It behaves like a high-grade soft tissue sarcoma and is associated with poor overall survival due to spreading to other parts of the body with recurrence after treatment also being very common. CCS neoplastic cells express the EWSR1-ATF1 fusion gene in most cases, with EWSR1-CREB1, EWSR1-CREM or EWSR1-DDIT3 fusion genes comprising a smaller subset of cases.

Prof Snelling leads the Tendon Seed Network chapter of the Human Cell Atlas, whose aim is to investigate the cell architecture of healthy human tendons. We have performed single-cell sequencing on several anatomically different tendon tissues across several healthy donors. Tendons have historically been thought of as acellular, however we have shown that tendons are composed of a diverse and rich cellular microenvironment. We are now applying these technologies to generate healthy tissue atlases of other joint-resident soft tissues including synovium and ligament

Considering that very little is known about the developmental origin of CSS, we hypothesise that CSS may develop from cells residing within the tendon. As such, we will leverage the reference maps of healthy musculoskeletal tissues with single-cell sequencing data of CSS that we will generate as part of this proposed project. We will cross reference these datasets of CSS and healthy soft tissues to identify whether the cellular origin of CSS is tendons or another tissue type. We will then determine the oncogenic drivers of CSS with the long-term goal of utilising this data to enable identification of novel therapeutics to treat this currently incurable cancer.

In collaboration with the Snelling group, work in the Cribbs lab focuses on developing novel single-cell technology and computational analysis frameworks that empower new modes of treatment for disease. Recently we have developed scCOLOR-seq[1], a method to overcome low basecalling accuracy making long-read single-cell transcriptomic sequencing highly accurate. This new technology enables us to measure translocations, alternative splicing, and allows variant calling. We have begun to apply this technology to understand the development of drug resistance in Sarcomas.

Research objectives and proposed outcomes
Our aim is to apply long-read single-cell sequencing technology to primary CCS patient samples and then generate computational models that will help us to identify the developmental origin and oncogenic drivers of this cancer.

Work package 1: Apply scCOLOR-seq to Clear Cell Sarcoma tumours.
The student will apply scCOLOR-seq to investigate the gene expression, isoform expression, translocations, and copy number variation within 10 CSS primary tumours. Specifically, the aim of this work package will be to identify genomic signatures that can provide us with a better understanding of the developmental origin on CCS.

Work package 2: Development of a computational analysis strategy to improve long-read single-cell sequencing.
Working simultaneously alongside work package 1, bespoke computational analysis pipelines will be written to help process the long-read sequencing data. The student will work alongside Dr Cribbs, who will provide extensive computational training, to develop skills in python and R programming, as well as software development. We have already developed generic long-read single-cell sequencing workflows (ref). The student will expand the development of this code with an emphasis on cross comparison analysis of our healthy tendon, ligament, synovium and soft tissue datasets and the long-read single-cell data generated in work package 1.

Work package 3: Develop and apply machine learning models to understand the genomic features that are important for developing Clear Cell Sarcoma.
Evaluate the accuracy and utility of a variety of unsupervised and supervised classification and machine learning algorithms (e.g. k-means/hierarchical clustering, linear discriminant analysis, support vector machines, Neural Networks and others) to identify features that are important for CCS pathogenesis. Specifically, we will develop a classifier model

using data (structural variation, isoform expression, gene expression) generated from the long-read sequencing experiments. Knowledge from this model will be used to identify features that drive the development of CSS and then generate a priority list of potentially druggable targets for functional validation.

Translational Potential

The stated aim of this project is to study the developmental origin of Clear Cell Sarcoma and identify drug targets for therapy. By its very definition, this work is likely to identify novel therapeutic intervention points within the development of Clear Cell Sarcoma.

We have extensive collaborations with several pharmaceutical partners, and we will utilise these interactions to explore the translational potential of targets.

Although CSS is rare, samples are already banked (50 frozen, 35 FFPE) which guarantees analysis can take place and we have successful pilot data showing high nuclei yields and robust sequencing. Our collaborators in London have been banking samples over a number of years and this ensures numbers are adequately powered to inform the study. Furthermore, we are continuing to collect CSS samples to provide additional validation samples. This methodology can be applied to other cancers and is a long-term goal from the work, but using CSS as an initial example due to the burden of disease and lack of diagnostic or treatment pathway.

Training opportunities

The student will receive training in cellular, molecular, and epigenetic biology for this project. This will involve wet-lab workflows for generating long-read single-cell sequencing data. Extensive training in computational biology will be provided so that the student can analyse their own data. Specifically, this will include software development, data analytics, statistics and computational pipeline development. Outside the lab, the student will be expected to attend regular seminars with high profile external speakers, journal clubs and training in presentation skills, scientific writing, and data management. As part of this project, you will collaborate and be co-supervised by Prof Pillay, a clinical pathologist at UCL who will provide samples for this study.

References

1. Philpott, M. et al. Nanopore sequencing of single-cell transcriptomes with scCOLOR-seq. *Nat Biotechnol* (2021).
2. Baldwin, M.J., Cribbs, A.P., Guilak, F. *et al.* Mapping the musculoskeletal system one cell at a time. *Nat Rev Rheumatol* **17,** 247–248 (2021).

4. How does macrophage marker F4/80 control peripheral tolerance during tumour progression? [1,2,3,4] – Prof. Kim Midwood

Primary Supervisor: Prof. Kim Midwood
Additional Supervisors: Professor Siamon Gordon and Dr Anja Schwenzer
Eligibility: All tracks are eligible to apply for this project.

Abstract
Our immune system detects tumors and activates cytotoxic T cells to destroy them. However, tumors can de-activate these cells, switching the immune response instead to become tolerant, helping cancer growth and spread. Drugs that reactivate cytotoxic T cells have revolutionized the treatment of people with cancer. However, this approach does not work for many patients, nor all types of tumor, and can be associated with severe side effects caused by the destruction of healthy tissues. Our recent data show how macrophages contribute to tumor progression, using the cell membrane marker F4/80 to communicate with, and switch on, tolerant T cells. However, whilst F4/80 is essential for tumors to survive and thrive, nothing is known about how this molecule works. We will investigate how F4/80 controls the immune axis in tumors. This will lead to a better understanding of how cancer hijacks the immune response for its own gain, and enable the design of new therapies that block this pathway, which are safer and effective in more patients than existing drugs.

Research objectives and proposed outcomes
Macrophages are terminally differentiated migratory leucocytes which sense physiologic and pathologic changes in their microenvironment through a variety of plasma membrane receptors that regulate their responses to maintain homeostasis (Fig.1A,B)[1]. In addition to specialised phagocytic receptors to recognize and clear senescent, apoptotic and necrotic cells and microbes, they are potent secretory cells able to nourish or destroy abnormal host cells and microbial targets, depending on their functional activation state. Tissue resident and recruited macrophages express adhesion molecules such as the F4/80 antigen, also known as EMR1 or ADGRE1, a widely used biomarker and the founder member of a family of 7 transmembrane G protein coupled receptors (GPCRs) that possess large extracellular epidermal growth factor- like (EGF) repeats. Human myeloid cells express a closely related EMR2 receptor, which is absent in the mouse, as well as an EMR1 homologue with distinct properties. In spite of extensive studies of its specific expression in the mouse, the role of F4/80 remains unknown. However, a major breakthrough in our understanding of its immunological function derived from studies using F4/80 knockout (KO) mice, which revealed this molecule to be dispensible for macrophage development, but responsible for peripheral tolerance. Required for Treg generation in vivo in response to a model antigen introduced into the anterior chamber of the eye [2], F4/80 expression is also essential for organ transplantation [3] and engraftment of Lewis lung cancer cells (Fig.1C-E). This project will explore the role of F4/80 in tumour-host interactions in vivo and identify cellular and extracellular binding partners of this adhesion GPCR.

Aim 1 will investigate the cellular and molecular basis of F4/80-mediated tolerance using a syngeneic, orthotopic breast cancer grafting model (well established at KIR)[4] in wild type or F4/80 null mice (maintained at the Dunn School). Immune profiling of tumor, spleen and lymph nodes during tumor growth using multicolour spectral flow cytometry (Aurora, KIR) will reveal F4/80 dependent changes in abundance, and activation/polarization status, across myeloid and lymphoid compartments. Multiplexed immunofluorescent imaging (GE CellDive, KIR) will reveal cell interaction partners of F4/80+ macrophages, and the tissue localization of these cellular networks. scRNA seq of sorted CD45+ cells from wild type and F4/80 knockout tumor bearing mice will reveal cell type-specific transcriptional changes associated with loss of F4/80, and pathway/interaction network analysis will highlight candidate effector molecules. Cellular interactions, and their contribution to F4/80-mediated tolerance, will be validated in vivo (e.g. by cell type or effector molecule depletion/blockade), and in vitro (e.g. using tumor-immune cell co-cultures) [4].

Aim 2 will identify extracellular binding partners of F4/80. More than 30 adhesion GPCRs exist, utilizing their sizable extracellular domains to form multimeric protein complexes of signalosome-like structures. Despite identification of extracellular ligands for other adhesion GPCRs, including the interaction of chondroitin sulphate (CS) and CD55 with close family members EMR2 [5] and CD97 [6] respectively, F4/80 remains an orphan receptor. F4/80 expression is elevated in specific murine breast tumor- associated macrophage subpopulations with enriched APC capabilities. These cells localize to extracellular matrix tracks that support cell infiltration into the tumor, whilst F4/80 low cells are restricted to the periphery (Fig.1F). Multiplexed imaging using a panel of matrix markers will identify tumor components that interact with F4/80+ cells in these tracks. In parallel, the adhesion of fetal liver macrophage cell lines from wild type and F4/80 knockout mice [7](Dunn School), to purified matrix molecules (e.g. tenascin-C (TNC), fibronectin, collagen type I/IV, osteopontin, CS)(KIR) as well as more complex matrices (e.g. matrigel, tumor derived cell free matrices) which better recapitulate the 3D environment of the tumor, will be assessed. Binding sites within F4/80 for ligands will be mapped as for CS-EMR2 [6] and downstream signaling examined.

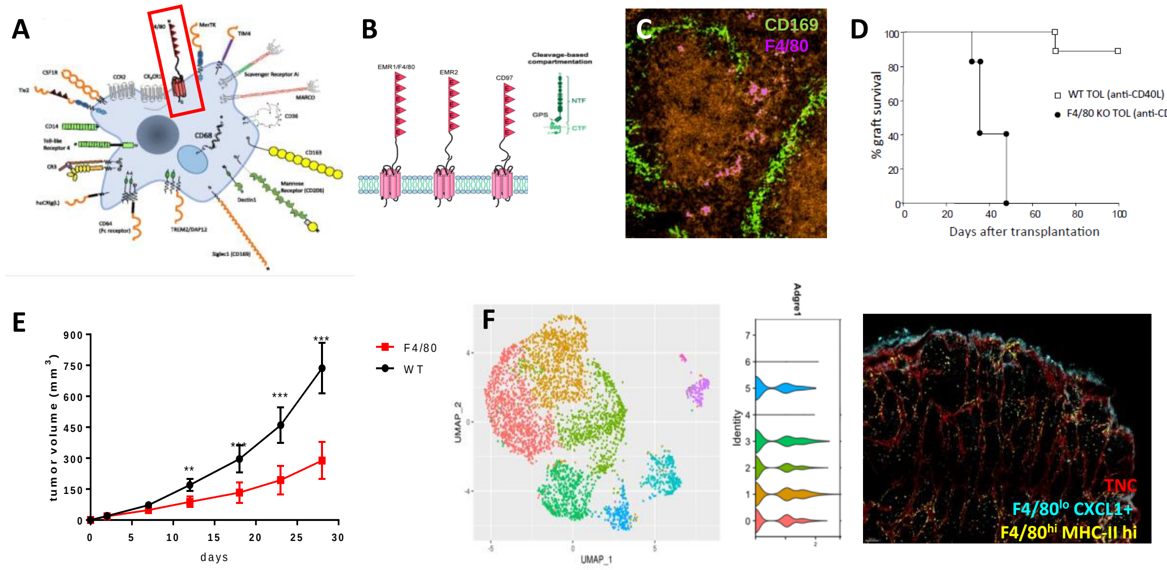

Figure 1. (A) Selected macrophage receptors. (B) Three of the EGF-TM7 family of adhesion GPCRs; EMR1, EMR2 and CD97. (C) F4/80+ APCs located in the spleen marginal zone 7d after antigen inoculation of the eye anterior chamber [2]. F4/80 deficiency abrogates tolerance during heart allograft [3](D) and Lewis cancer cell transplant (unpublished)(E). (F) F4/80 expression is elevated in MHC^{hi} macrophage subpopulations localized exclusively to matrix-rich tracks in murine breast tumors (our unpublished data).

Translational potential

Macrophages are an integral component of tumours, responding to, and in turn, influencing the malignant cells, as well as all stromal and other immune cellular and extracellular components of the tumour microenvironment. Their interactions with the extracellular matrix could affect their own growth, adhesion, migration and activation, as well as of the malignant cells and metastases. We need to learn more about their polarization, and the switch from trophic to cytotoxic potential, crucial in the tumour-host interaction. This project will establish some of the basic principles for further translation to human cancer. Among the EGF-TM7 family CD97 and EMR2 have been investigated in a range of human cancers, with, for example, changes in the cellular compartmentalization of EMR2 correlating with poor prognosis in breast cancer [8]. F4/80 has been neglected hitherto. Evidence that it mediates peripheral tolerance, which can be abrogated by targeting F4/80, makes this a compelling model for further research in mouse models of cancer. Moreover, study of the autoproteolyic cleavage of these adhesion GPCRs (Fig1B), may also be relevant to shedding of soluble receptor in vivo, which could contribute to cancer pathogenesis and to diagnosis through its presence in plasma or other body fluids [9].

Training opportunities

The student will be trained in techniques including: in vivo tumor models, immune cell isolation/activation, 2D & 3D matrix adhesion assays, multiplexed tissue imaging, spectral flow cytometry, RNA seq dataset generation and bioinformatic analysis in mouse and human pathology

References

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5. Chronic infection, host immunity, and cancer risk [3,4] – Ling Yang

Primary Supervisor: ling Yang
Additional Supervisors: Christiana Kartsonaki
Eligibility: All tracks are eligible to apply for this project.

Abstract
Worldwide, chronic infection of certain pathogens (i.e. virus, bacteria and parasite) is responsible for more than 2 million new cancer cases each year, with about a third of these cases occur in China.[1] Although the aetiological roles of several common pathogens (e.g. *H. pylori*, HBV, HCV, and HPV) for certain cancers are well established, questions remain about their relevance for other cancers and the role of other pathogens (e.g. human herpes virus) in cancer development. Previous studies have been often constrained by small sample sizes and investigation of a single pathogen with a single cancer. Consequently, uncertainty remains about the roles of common chronic infections in cancer development and progression.[2] It is also unclear as to whether there are important interactions between infections and other lifestyle/behavioural risk factors for cancer, and how genetic variants in both host (e.g. human leukocyte antigen; HLA) and pathogen and their interactions influence host immune response and susceptibility to and progression of infection, as well as subsequent cancer risk. For example, variability in HLA class I or II type antigens that affect the cellular immune response to HPV may partially explain why only a minority of infected women develop cervical cancer.[3]

The China Kadoorie Biobank (CKB) included 0.5 million Chinese people during 2004-08 from 10 different areas across China, with different types of information collected by questionnaire and physical measurements, and with long-term follow-up of health outcomes for each participant by the linkage with national death, disease registries and nationwide health insurance systems. So far, we have collected more than 70,000 deaths and over 1.5 million hospitalization events for >5000 different diseases. In subsets of CKB participants, we also had genetic and other biomarkers (e.g. inflammation) measured.

Infectious agent	Antigen
Background marker	GST
HSV1	gG
HSV2	mgG unique
VZV	gE / gI
EBV	VCA p18, EBNA-1, ZEBRA, EA-D, BFRF1, BGLF2, BXLF1
CMV	pp150 Nter, pp 52, pp 28
HHV7	U14
HHV6	IE1B trunc, IE1A trunc
HBV	HBc, HBe
HCV	Core, NS3
Toxopl. gondii	p22, sag-1
HTLV-1	gag, env
HIV-1	gag, env
HPyV	BK VP1, JC VP1, MCV VP1
HPV	HPV 16 L1, HPV 16 E6, HPV 16 E7, HPV 16 E1, HPV 16 E2, HPV 18 L1, HPV 18 E6, HPV 18 E7
C. trachomatis	pGP3
H. pylori	CagA-N, GroEL, OMP, VacA-C, Catalase, HcpC, HP0305, HopA
Coxiella burnetii	CBU_1910 Com1

Based on this CKB cohort, we are conducting a large case-cohort study including all incident site-specific cancer cases in China (>30,000) and a randomly selected sub-cohort of participants (~10,000) to assess and quantify the role of different infectious pathogens in various cancers development. In collaboration with UK Biobank and the German Cancer Research Centre (DKFZ), a CKB custom-designed multiplex serology [4] panel has been developed and been used to detect and quantify 47 antibodies for 17 infectious pathogens (see details in Table), selected for their cancer relevance and will enable population-specific variation in infections to be assessed in relation to different cancers. Moreover, in collaboration with the Wellcome Centre for Human Genetics, we are sequencing HBV and HCV viral genomes from ~5,000 CKB HBV infected participants, half of whom who developed incident HCC during follow-up and the other half who did not. Genome-wide association studies (GWAS) and genome-to-genome analysis will be used to investigate host-virus interactions and genotype-phenotype relationships.[5]

These, together with available lifestyle, physical measurements, medical history, other biomarkers and health outcome data, will enable comprehensive assessment of causal roles of multiple chronic infections in aetiology of site-specific cancers and certain other diseases.

The proposed DPhil project will be based on this ongoing infection and cancer programme and the specific research proposal will be developed according to the student's interests and aptitude.

Research objectives

The specific lines of investigation covered by this DPhil project will depend on the student's interests and previous training, and may be involved within following key areas of work: (1) to examine the associations of chronic infection of particular pathogens with risks of all or certain site-specific cancers and estimate the infection-related cancer burden; (2) to explore the role of host immune system genetics (e.g. HLA) in susceptibility to specific types of chronic infection and cancer; (3) to establish the value of serological markers, in combination with other lifestyle and genetic risk factors, in predicting the risk of infection-related cancers.

By the end of the DPhil, the student will be competent to review the literature, to plan, undertake and interpret analyses of large-scale data, and to report research findings, including 3-5 publications in peer-reviewed journals and presentation at conferences.

Translational potential

This project will lead to improved understanding about the aetiological role of infectious pathogens in cancer development, help identify high risk individuals for early detection/mass-screening and targeted cancer treatment and inform policy makers to develop and implement suitable strategies for cancer prevention locally and globally.

Training opportunities

The student will be based within the CKB research group in Nuffield Department of Population Health. There are excellent facilities and a world-class community of population health, data science and genomic medicine researchers. There will be in-house training opportunities in epidemiology, statistics, genetics, and bioinformatics and if necessary attendance at relevant courses.

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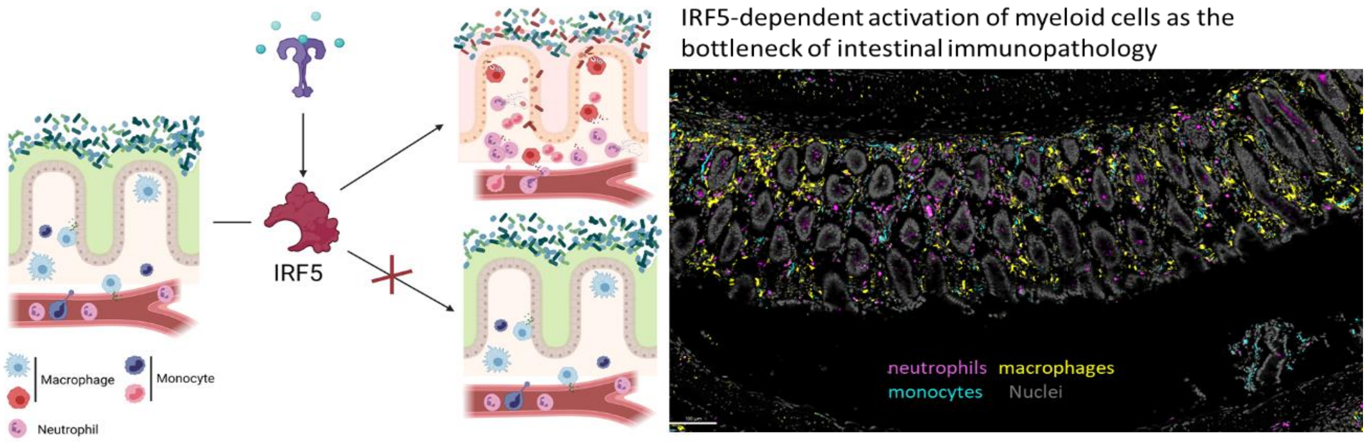
6. Myeloid cells in sustained intestinal inflammation and colorectal cancer: the role of IRF5 [1,2,3]– Prof. Irina Udalova

Primary Supervisor: Prof. Irina Udalova
Additional Supervisors: Prof. Fiona Powrie
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Abstract
Dysregulation of the intestinal immune system can lead to inflammation, which may progress to colorectal cancer (CRC)n [1]. Myeloid cells are playing a central role in maintenance of homeostasis, initiation of inflammation, restoration of tissue upon injury and mediation of chemoresistance in tumours. Integrating cues of their immediate tissue microenvironment. Interferon Regulatory Factor 5 (IRF5) has been identified to act as a master transcription factor in myeloid cells that controls both acute and chronic inflammation and is protective in pathogen clearance [2,3,4]. IRF5 has been shown to regulate macrophage transcription of pro-inflammatory mediators, monocytes differentiation into pro-inflammatory macrophage phenotype, and more recently, neutrophil effector responses without compromising neutrophil maturation processes or their ability to enter into the tissue [5]. Our preliminary data suggest that IRF5 function in myeloid cells is indeed a key element in controlling the onset of intestinal inflammation and pathology, but its role in resolution of inflammation or cancerogenesis is not well understood. Of relevance, IRF5 has also been identified as a DNA-damage sensor, highlighting a potential beneficial role in CRC [6]. Both tumour-associated macrophages (TAMs) and tumour-associated neutrophils (TANs) appear to play a major role in cancer progression or hinderance [7]. Therefore, dissecting the molecular mechanisms orchestrating neutrophil, monocyte and macrophage function is crucial for identification of treatment options for both inflammatory bowel diseases (IBD) and CRC.



Research Objectives
First, we will use advanced imaging and spatial transcriptomic analyses to compare myeloid cell heterogeneity in the colon in resolution of inflammation using the *Helicobacter hepaticus* and anti-IL10R (Hh + aIL10R) colitis model [2] with that in microbe driven colitis associated cancer (CAC) models [8], characterised in the Powrie group; as well as during the onset and peak of inflammation, characterised previously in our lab. It was suggested that localisation of macrophages and neutrophils within the tumour microenvironment might be a crucial determinant of their function [9]. Therefore, using already generated reporter strains (e.g. CX3CR1-GFP x Ly6G-Tomato) we will assess the localisation of different macrophage and neutrophil subsets and their interaction cell-cell contacts, which could also provide further information about their function and potential targeting. This will help identify molecular targets in shaping their phenotype and directing towards resolution rather than progression of inflammation into cancer development.
Second, we will assess the role of IRF5 in myeloid cells. Previous work in the lab has profiled IRF5-dependent inflammatory CD11c+ macrophages at peak of inflammation using scRNA-seq [2]. New data suggest the role for IRF5 in driving neutrophil-dependent inflammation. Based on this work, we aim to utilise already generated targeted mouse models (CX3CR1-cre ER2 IRF5 fl/fl; CCR2-mKate-cre ER2 IRF5 fl/fl; Ly6G-cre IRF5 fl/fl) to understand the beneficial vs pathologic role of IRF5 in resolution of inflammation. We hypothesize that lack of IRF5 in macrophages may be beneficial as macrophages are being polarised towards a tissue-regenerating phenotype. Furthermore, the T cell pool was shifted at peak of inflammation, which might be a result of different T cell priming by IRF5-proficient and deficient macrophages. The lack of IRF5 in neutrophils may be detrimental as neutrophils can be involved in priming barrier cell repair and/or regenerating the extra cellular matrix around the perturbances.

Third, the role of TAMs and TANs in CRC is yet unclear with various studies suggesting both detrimental and beneficial effects. Investigation of the differences in T cell priming of IRF5-deficient macrophages and/or neutrophils will also offer insight of the interplay of IRF5 in innate immune cells with the adaptive immune system in both resolution and CRC. Thus, in addition to inhibition of IRF5 (as above) we would also consider stimulating IRF5 specifically at tumour sites might improve anti-cancer immunity [10]. This could be achieved by targeted delivery of adenoviral vector expressing IRF5 (overexpression) or inhibition of IRF5 activation through phosphorylating kinase inhibition [11] in CAC models.

Translational potential

T cell immunity, which is beneficial in tumours, is undermined by immunosuppressive myeloid cells, of which a subset of TREM2+ macrophages have been identified as a potential target in tumours [12]. Understanding the role of macrophages as pivotal cells in the resolution of inflammation as well as progression of inflammation into CRC will help shaping specific therapies targeting macrophages. IRF5 also plays a crucial role in mediating monocyte recruitment and their differentiation into pro-inflammatory macrophages, as well as in effector neutrophil functions, during intestinal inflammation and may therefore be central during resolution and cancer development. Moreover, the inhibitor of IRF5 activation pathway may prove beneficial for inflammation-induced cancer.

Training opportunities

The student will be trained in the Hh + aIL10R colitis and CAC models as well as in basic immunology techniques like flow cytometry, RT-qPCR and *in vitro* cultures to analyse the outcomes. Furthermore, insights and potential guided analysis of single-cell RNA sequencing as well as cutting-edge microscopy and spatial transcriptomics (GeoMx and CosMx Nanostring platforms) to define the localisation of macrophage subsets within the tumour microenvironment will be made available.

References

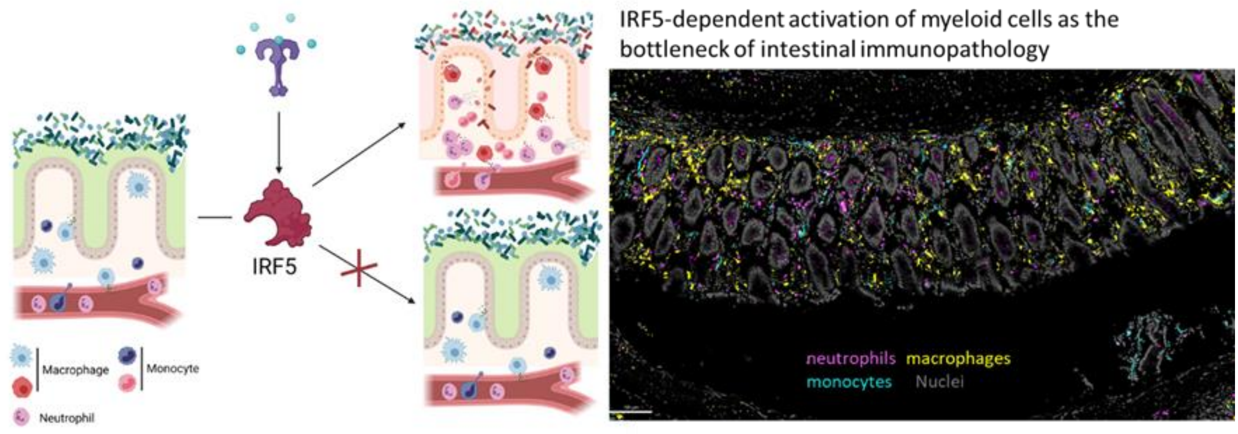
1. Mantovani, A *et al*. Cancer-related inflammation. *Nature* 454, 436–444 (2008). **2**. Corbin, A. L. *et al.* IRF5 guides monocytes toward an inflammatory CD11c + macrophage phenotype and promotes intestinal inflammation. *Sci. Immunol.* 6085, 1–16 (2020). **3**. Weiss, M. *et al.* IRF5 controls both acute and chronic inflammation. *Proc. Natl. Acad. Sci. U. S. A.* 112, 11001–11006 (2015). **4**. Pandey, S. P., Yan, J., Turner, J. R. & Abraham, C. Reducing IRF5 expression attenuates colitis in mice, but impairs the clearance of intestinal pathogens. *Mucosal Immunol.* 12(4):874-887 (2019). **5**. Khoyratty, T. E. & Ai, Z. Distinct transcription factor networks control neutrophil-driven inflammation. *Nat. Immunol.* 22(9):1093-1106 (2021). **6**. Hu, G. et al. Signaling through IFN regulatory factor-5 sensitizes p53-deficient tumors to DNA damage-induced apoptosis and cell death. *Cancer Res.* 65, 7403–7412 (2005). **7**. Quail, D. F. *et al.* Neutrophil phenotypes and functions in cancer: A consensus statement. *J. Exp. Med.* 219, 39 (2022). **8**. Kirchberger, S. *et al.* Innate lymphoid cells sustain colon cancer through production of interleukin-22 in a mouse model. *J. Exp. Med.* 210, 917–931 (2013). **9**. Caprara, G., Allavena, P. & Erreni, M. Intestinal Macrophages at the Crossroad between Diet, Inflammation, and Cancer. *Int. J. Mol. Sci.* 21, 4825 (2020). **10**. Byrne, A. J. *et al.* A critical role for IRF5 in regulating allergic airway inflammation. *Mucosal Immunol.* 10, 716–726 (2017). 11. Ryzhakov et al. Defactinib inhibits PYK2 phosphorylation of IRF5 and reduces intestinal inflammation. *Nat Commun* 12(1):6702 (2021). **12**. Molgora, M. *et al.* TREM2 Modulation Remodels the Tumor Myeloid Landscape Enhancing Anti-PD-1 Immunotherapy. *Cell* 182(4)**,** 886-900 (2020).

7. Cancer vaccination for precision prevention of Lynch Syndrome [1,2,3]– Prof. Simon Leedham & Assoc Prof. David Church

Primary Supervisor: Prof. Simon Leedham & Assoc Prof. David Church
Additional Supervisors: Professor Sarah Blagden
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project

Abstract

Dysregulation of the intestinal immune system can lead to inflammation, which may progress to colorectal cancer (CRC)n [1]. Myeloid cells are playing a central role in maintenance of homeostasis, initiation of inflammation, restoration of tissue upon injury and mediation of chemoresistance in tumours. Integrating cues of their immediate tissue microenvironment. Interferon Regulatory Factor 5 (IRF5) has been identified to act as a master transcription factor in myeloid cells that controls both acute and chronic inflammation and is protective in pathogen clearance [2,3,4]. IRF5 has been shown to regulate macrophage transcription of pro-inflammatory mediators, monocytes differentiation into pro-inflammatory macrophage phenotype, and more recently, neutrophil effector responses without compromising neutrophil maturation processes or their ability to enter into the tissue [5]. Our preliminary data suggest that IRF5 function in myeloid cells is indeed a key element in controlling the onset of intestinal inflammation and pathology, but its role in resolution of inflammation or cancerogenesis is not well understood. Of relevance, IRF5 has also been identified as a DNA-damage sensor, highlighting a potential beneficial role in CRC [6]. Both tumour-associated macrophages (TAMs) and tumour-associated neutrophils (TANs) appear to play a major role in cancer progression or hinderance [7]. Therefore, dissecting the molecular mechanisms orchestrating neutrophil, monocyte and macrophage function is crucial for identification of treatment options for both inflammatory bowel diseases (IBD) and CRC.



Research objectives

First, we will use advanced imaging and spatial transcriptomic analyses to compare myeloid cell heterogeneity in the colon in resolution of inflammation using the *Helicobacter hepaticus* and anti-IL10R (Hh + aIL10R) colitis model [2] with that in microbe driven colitis associated cancer (CAC) models [8], characterised in the Powrie group; as well as during the onset and peak of inflammation, characterised previously in our lab. It was suggested that localisation of macrophages and neutrophils within the tumour microenvironment might be a crucial determinant of their function [9]. Therefore, using already generated reporter strains (e.g. CX3CR1-GFP x Ly6G-Tomato) we will assess the localisation of different macrophage and neutrophil subsets and their interaction cell-cell contacts, which could also provide further information about their function and potential targeting. This will help identify molecular targets in shaping their phenotype and directing towards resolution rather than progression of inflammation into cancer development.

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Translational potential

T cell immunity, which is beneficial in tumours, is undermined by immunosuppressive myeloid cells, of which a subset of TREM2+ macrophages have been identified as a potential target in tumours [12]. Understanding the role of macrophages as pivotal cells in the resolution of inflammation as well as progression of inflammation into CRC will help shaping specific therapies targeting macrophages. IRF5 also plays a crucial role in mediating monocyte recruitment and their differentiation into pro-inflammatory macrophages, as well as in effector neutrophil functions, during intestinal inflammation and may therefore be central during resolution and cancer development. Moreover, the inhibitor of IRF5 activation pathway may prove beneficial for inflammation-induced cancer.

Training opportunities

The student will be trained in the Hh + aIL10R colitis and CAC models as well as in basic immunology techniques like flow cytometry, RT-qPCR and *in vitro* cultures to analyse the outcomes. Furthermore, insights and potential guided analysis of single-cell RNA sequencing as well as cutting-edge microscopy and spatial transcriptomics (GeoMx and CosMx Nanostring platforms) to define the localisation of macrophage subsets within the tumour microenvironment will be made available.

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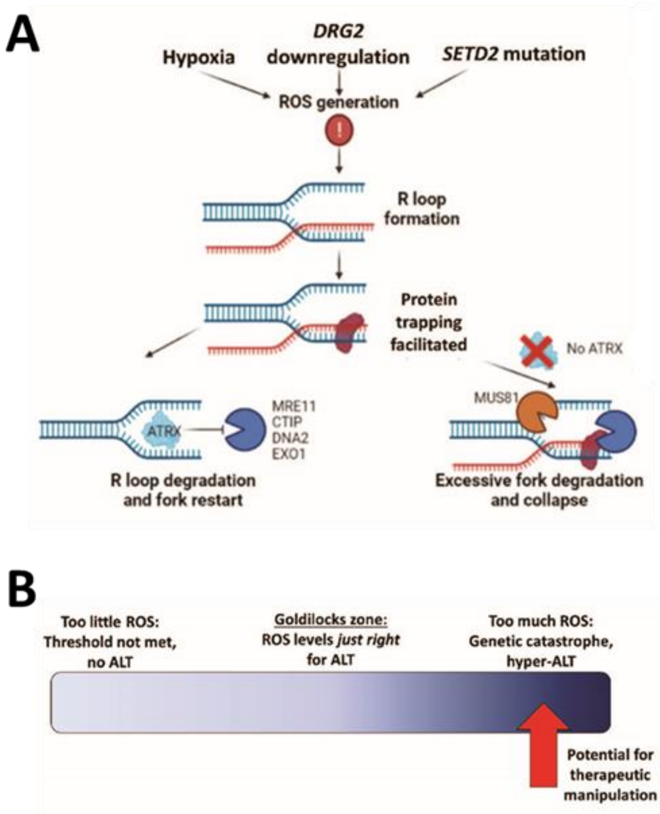
8. Therapeutic manipulation of reactive oxygen species in ATRX-deficient cancers [1,2,3] – Prof. Ester Hammond

Primary Supervisor: Prof. Ester Hammond
Additional Supervisors: Dr Anna M. Rose
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Abstract

Telomere maintenance is an essential cancer hallmark, allowing malignant cells to divide without limit. One major telomere maintenance mechanism is called Alternative Lengthening of Telomeres (ALT). The ALT-pathway is particularly prevalent in aggressive brain cancers (such as high-grade glioma (>40%) and astrocytoma (90%)), as well as cancers of mesenchymal origin, such as osteosarcoma [1]. The central genetic event underpinning ALT-pathway activation is loss of ATRX [2]. In addition to ATRX loss, ALT-pathway activation requires another factor. Our recent work demonstrated that this second factor is excessive accumulation of reactive oxygen species (ROS) in the tumour microenvironment, which could be due to hypoxia, concurrent gene mutation and/or redox gene dyrsregulation. Elevated ROS levels lead to trapping of DNA-interacting proteins, which subsequently causes replication fork stalling and ALT-pathway activation [3]. ATRX protein is essential for fork re-start and so, in the absence of ATRX, there is aberrant downstream processing of stalled forks [4,5]. This aberrant processing produces DNA double-strand breaks, the genetic substrate for ALT-telomere elongation (**Figure A**).



Crucially, however, there is a "Goldilocks zone" of ROS for cells lacking ATRX: too little ROS, and the threshold of ALT-pathway activity is not met, and telomere synthesis is not triggered. Excessive ROS levels, though, lead to genetic catastrophe and cell death, as ATRX-null cells have intrinsic vulnerability to DNA damage due to the many roles of ATRX in genome integrity. There is, however, a level at which ROS are "*just right*", where ATRX-deficient cells can leverage ROS-induced trapping of proteins to allow telomere maintenance and limitless cell division (**Figure B**). Therapeutic manipulation of ROS levels to outside of the "Goldilocks zone" provides a novel approach to treatment for ATRX-deficient ALT cancers.

The central aim of this project is to exploit the ROS-dependency of ALT cancer cells for therapeutic benefit in ATRX-deficient tumours. In particular, we will explore the potential of novel redox modulating agents i.e. those that either increase ROS directly or, indirectly by decreasing anti-oxidant capacityin sensitization of ATRX-deficient cancer cell lines and animal models to standard of care chemotherapy and radiotherapy. As well as assessing the efficacy of these agents, we will study the molecular mechanisms and underpinning cell biology, allowing a deeper understanding of the role of ROS in ATRX-deficient ALT cancers.

Research objectives

The rationale for this study is that whilst ATRX-mutation drives the evolution of ALT-cancers, it also provides a unique therapeutic opportunity: ATRX is ubiquitously-expressed with roles in many cellular processes and so cells lacking ATRX have vulnerabilities which can be therapeutically exploited. In this project, we will explore the genetic and cellular perturbations in ALT-cancers, in particular, the role of abnormal reactive oxygen species (ROS) metabolism. Prof. Hammond is an expert in redox and hypoxia biology in cancer. Dr Rose is an expert in ATRX cell biology and the ALT pathway, with clinical expertise in paediatric oncology. Our pilot work demonstrated that ALT-positive gliomas have strong dysregulation of redox pathway genes, highlighting the potential role of ROS. Further, treatment of ATRX-deficient cells with ROS-generating agents induced ALT-pathway activity in non-ALT cell lines. Excessive ROS led to trapping of proteins on DNA, leading to the formation of abnormal DNA lesions. Further, we found that both the accumulation of trapped proteins and induction ALT-pathway was dependent on the accumulation of R-loops, RNA:DNA hybrid structures. Our preliminary data also suggested that pre-treatments which elevate ROS (such as silencing of SOD1 gene) sensitised ATRX-deficient cells to camptothecin, through induction of hyper-ALT. Camptothecin derivatives – such as irinotecan/topotecan – are widely used in the treatment of brain cancers. In this project, we will greatly expand this preliminary data by using ROS-generating treatments to sensitise a range of ALT brain cancer cell lines to various chemotherapeutic agents (e.g. etoposide, camptothecin-derivatives, PARP-inhibitors). Through the collaboration with Dr Monica Olcina, we will also assess the role of such pre-treatments in radiosensitisation, including in pre-clinical murine models, as appropriate. Radiotherapy itself is known to generate ROS and, as such, this could represent a novel approach to hyper-ALT induction

Translational potential
Development of novel therapeutics for ATRX-deficient cancers is an urgent area of clinical unmet need [6,7]. The outcomes for ALT-cancers is very poor, with little progress made in survival in over 50 years. The work in this project is hypothesis-driven and will generate pre-clinical data that will be critical in informing future clinical trials and translational work. The insights into gene dysregulation, telomere dysfunction and genome stability will clarify the pathways involved in ALT-cancer biology, which is the first critical step in developing targeted therapies. The project will involve the opportunity to conduct cell and animal studies using novel ROS-inducing agents, assessing their ability to sensitise ATRX-deficient cells to traditional standard-of-care chemotherapy and radiotherapy. These assays will hopefully lead to future early-phase clinical trials of the novel agents. Further, our work into chemosensitisation and radiosensitisation will align directly with our groups wider work into oncolytic virus delivery of synthetic lethal shRNA molecules to ATRX-deficient cells.

Training opportunities
Prof. Hammond, Dr Rose and Dr Olcina have worked together collaboratively for the past 2-years, developing a new and exciting interdisciplinary collaboration. They have a strong track record for supervising DPhil, MSc and BSc students. This project offers the opportunity to join a well-funded, collaborative and interdisciplinary team. The student will be based jointly in the Hammond lab (Department of Oncology) and Rose group (Department of Paediatrics), with strong links to the Olcina group. The student will have the opportunity to learn a wide range of molecular and cell biology techniques including tissue culture, protein analysis, qRT-PCR, c-circle assay, immunoflouresnce and microscopy, RADAR assay, gene silencing and overexpression, use of radiation sources, hypoxia chambers, and *in vivo* mouse work.

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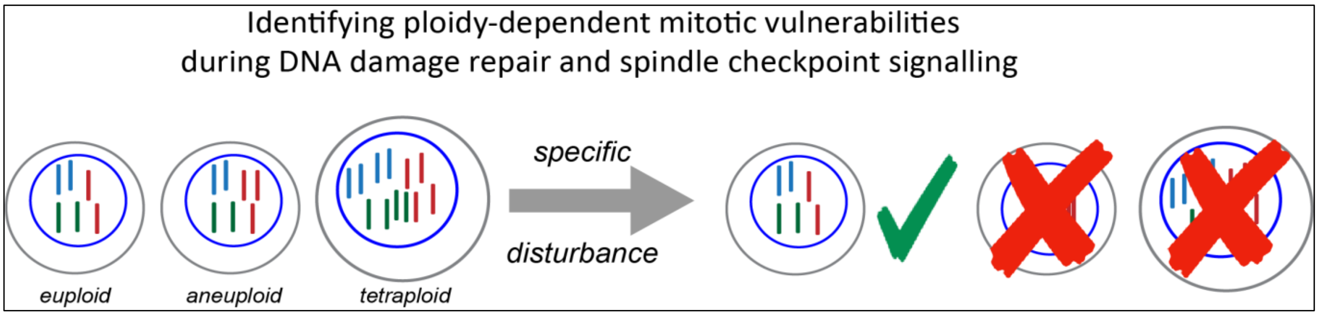
9. Identifying ploidy-dependent mitotic vulnerabilities during DNA damage repair and chromosome segregation [1,2,3] – Prof. Ulrike Gruneberg

Primary Supervisor: Prof. Ulrike Gruneberg
Additional Supervisors: Prof. Monika Gullerova
Eligibility: Track 1,2 and 3 students are eligible to apply for this project.

Abstract
Faithful cell division ensures the correct segregation of the genetic material over multiple generations. Failure of this process may result in cells of abnormal ploidy, such as aneuploidy or tetraploidy, caused by whole-genome doubling. Ploidy changes have been implicated in enabling cancer evolution to take place and are thus considered a driving force for tumorigenesis. On the other hand, ploidy changes may impart specific vulnerabilities to tumour cells. Recent reports identified the spindle assembly checkpoint, the key quality control mechanism during mitosis, as well as DNA damage repair dealing with replication stress, as such vulnerabilities [1-4]. These insights could potentially be exploited for therapeutic use. We propose to examine the precise mechanisms and effects of disrupting DNA damage repair, the spindle assembly checkpoint and other mitotic control mechanisms in non-transformed and transformed diploid and tetraploid cells with the aim of identifying and characterising specific ploidy-dependent susceptibilities.



Research objectives
Tetraploidy and aneuploidy have both been shown to promote tumorigenesis [5,6]. To understand the molecular biology underpinning these properties, we will compare euploid non-transformed MCF10A breast and hTert-immortalised retinal epithelial cells (hTert-RPE1) with near-diploid tumour cells HCT116 and highly aneuploid tumour HeLa cells, as well as tetraploid derivatives of hTert-RPE1, MCF10A and HCT116. These cell lines constitute an accepted system to probe the relationship between ploidy and dependence on mitotic control mechanisms [2]. Tetraploid cells will be generated by induction of cytokinesis failure and FAC sorting for tetraploid DNA content, as already established in the lab. Recent research in both yeast and mammalian cells has shown that tetraploid and aneuploid cells are more sensitive to replication stress during S-phase as well as mitotic aberrations during the cell division process, giving rise to the idea that these vulnerabilities could be exploited therapeutically [2-4].

Together with our collaborator Monika Gullerova, we will use our combined expertise in spindle assembly checkpoint and DNA damage repair analysis to carry out a targeted comparative analysis of perturbing these processes in cells of different ploidies with the aim of identifying disturbances which affect aneuploid or tetraploid cells more than their euploid counterparts. In particular, our previous research in the Gruneberg lab into the regulatory roles of distinct kinase-phosphatase modules at different mitotic transitions is likely to identify experimental situations which affect aneuploid cells more negatively than euploid cells [7-10]. To characterise these situations, we will compare the sensitivity of cell lines with different ploidies to different phosphatase depletions/degron-tags (siPP1 & $PP1^{dTag}$, siPP2A-B56, siPP2A-B55) and analyse timing and success of cell cycle progression, error correction proficiency and spindle assembly checkpoint competence using established assays. For DNA damage repair analysis, we will test the repair competence of cells with different ploidies using various experimental approaches such as non-homologous-end-joining and homologous recombination reporter cell lines, comet assay, clonogenics and gH2AX clearance [11,12]. Any differences between aneuploid and diploid cells will be followed up by a detailed analysis of the molecular biology underpinning these, using fluorescent markers that we have already in the lab, including the key spindle checkpoint kinase MPS1, the attached-kinetochore marker astrin, and fluorescently-tagged cyclin B as a general marker of cell cycle progression, to assess the success of the different stages of chromosome segregation.

Translational potential

Aneuploidy and tetraploidy have long been recognised as drivers of tumorigenesis. As these are states that are largely specific to cancer cells, there is an impetus to use the vulnerabilities that are created by these abnormal ploidies to specifically eradicate tumour cells. We postulate that specific interference with DNA damage repair or alterations to the kinase-phosphatase balance orchestrating mitotic progression will be lethal to aneuploid tumour cells but not their euploid untransformed counterparts. Our characterisation of the effect of these disturbances on cells with abnormal ploidy will thus be of significant therapeutic value.

Training opportunities

Students will receive comprehensive training in molecular biology, classical protein biochemistry, assays to analyse DNA damage repair and spindle assembly checkpoint proficiency, cutting edge fixed and live cell imaging techniques as well as quantitative image analysis methods.

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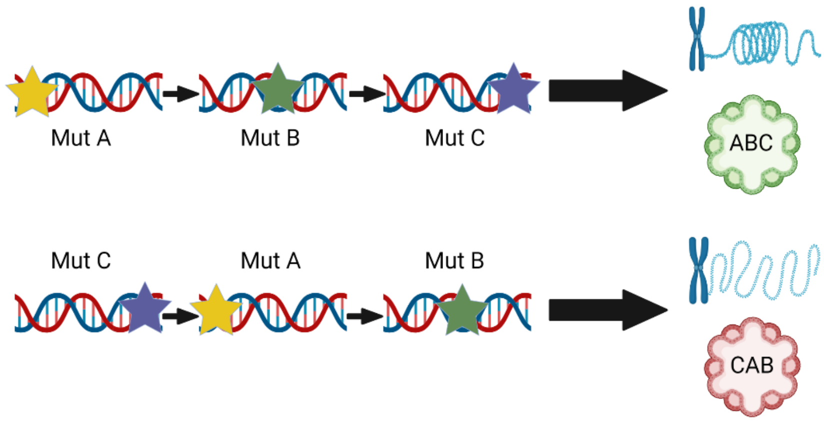
10. The role of mutational order in colorectal cancer evolution, epigenetics and response to therapy [1,2,3] – Prof. Simon Buczacki

Primary Supervisor: Prof. Simon Buczacki
Additional Supervisors: Dr. Dan Woodcock
Eligibility: Track 1,2 and 3 students are eligible to apply for this project.

Abstract

Cancer development is characterised by the sequential acquisition of somatic mutations in DNA. Leveraging our expertise in gene engineering (Chan et al. 2023, Chan et al. 2023) of human intestinal organoids (3D mini guts), our group has recently shown for the first time in solid organ cancers, that the order in which mutations are acquired results in unexpected changes to phenotype, gene expression and response to drug therapy (**Fig 1**) (Chan et al. *Manuscript Submitted*). These heretical data identify that alterations in DNA structure (chromatin) and accessibility appear key in determining the functional consequences of changes to mutational order. Our findings establish mutational order as a novel route to both tumour heterogeneity and resistance to targetted agents. This project maximises on our group's expertise in gene engineering, organoid culture and multiomics to advance our recent findings to develop clinically applicable multiomic signatures of mutational order (SMOs) that may better help predict tumour biology and response to therapy.



Research objectives

WP1 – Production and multiomic characterisation of CRISPR-engineered switched colon organoid models containing the main truncal mutations of colorectal cancer in distinct orders. Using protocols established in our group, the student will generate CRISPR-Cas9 engineered organoids containing mutations in *APC, KRAS, P53, SMAD4* and *PIK3CA* (**Fig 2**). Isogenic organoid models will be generated with mutations placed in reverse and alternate orders. Confirmation of targetting will be performed using Sanger sequencing and western blotting. Organoid models will be analysed using RNA sequencing (gene expression) and ATAC sequencing (chromatin accessibility). Live cell imaging, immunofluorescence and electron microscopy (scanning and transmission) will be performed to characterise phenotypes. Selected models will also be anlaysed using single cell RNA sequencing to establish changes in cell fate and identity.

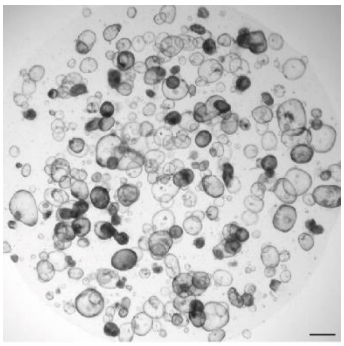


Figure 2. CRISPR-Cas9 mutated human colon organoids

WP2 – Drug screen analysis of CRISPR-engineered switched organoid models. Our group has an established pipeline for drug screening of cancer organoids. The student will ultilise this pipeline to perform drug screening using both standard-of-care colorectal cancer treatments and novel compounds in engineered organoids and isogenic-switched models. Organoids will be assessed for viability and characterised using a live cell imaging platform.

WP3 – Integration of experimental multiomic analyses to generate signatures of mutational order (SMOs). Data generated in WP1 will be integrated using advanced bioinformatic techniques to generate SMOs. SMOs will be validated on publically available data sets to identify tumours where mutational order can be inferred from SMO presence. SMOs will be applied to identify differential response to adjuvant treatment regimes and variations in clinical outcomes.

Translational potential

Until very recently due to technological constraints, the ability to study effects of alterations in mutational order have been near impossible. Our pioneering work in this new area of cancer biology has identified that changes in mutational order unexpectedly generate not only profound variations in cell behaviour and stem cell dynamics but also lead to differential responses to chemotherapies. Whilst there are multiple routes to drug resistance and sensitivities, the impact of mutational order on this remains completely unexplored. This project therefore has the potential to generate novel mechanistic insights and new putative drug targets to improve chemotherapy efficacy.

Training opportunities

The student will be directly supervised by Prof. Simon Buczacki (Tumour Evolution and Cell Identity Laboratory). All students in the group have a weekly one to one with Prof. Buczacki. We have a weekly lab meeting where the student will present their data and discuss findings to the wider group and collaborators. There is common cross-fertilisation of ideas and expertise within the group to foster a caring and supportive lab environment. The group's philosophy is to constantly challenge traditional dogma and address the 'big' questions in cancer and stem cell biology. The student will be trained in a wide range of wet and dry lab techniques by members of the group expert in these areas. The group is fortunate to have an embedded post-doctoral bioinformatician with significant expertise in multiomic analyses (Dr Amit Mandal). The second supervisor, Dr Dan Woodcock is an expert in cancer evolution and multiomic data integration. Dr Woodcock's expertise as second supervisor will be integral to many aspects of the project especially WP3.

Wet lab: Human tissue processing, organoid culture (normal and tumour), CRISPR-Cas9 gene engineering, NGS (RNAseq,scRNAseq, ATACseq), RT-PCR, western blotting, Sanger sequencing, flow cytometry and microscopy (confocal, live cell and EM).

Dry lab: Multiomic data (processing, analysis, integration), coding (R, Python) and big data handling.

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11. Spatial resolution of the human transcriptome during gastrointestinal tumorigenesis – [1,2,3,4] Dr. Francesco Boccellato

Primary Supervisor: Dr. Francesco Boccellato
Additional Supervisors: Jan Bornschein
Eligibility: All tracks are eligible to apply for this project.

Abstract
The gastrointestinal mucosa is organised in invaginations called glands in the stomach and crypts in the colon. How cancer arises from these invaginations is still unclear, but before cellular transformation occurs, the analysis of early mucosal aberrations in biopsies enables the detection of pre-cancerous conditions. Tissue response to stress, toxic dietary compounds, infections and inflammation, might alter the microenvironment posing the mucosa at risk of malignant transformation. We plan to analyse pre-cancerous conditions of the gastrointestinal tract to understand why they have a different tissue configuration compared to the normal.

Growth factors and morphogens shape the tissues during embryogenesis, and they are probably responsible of its homeostasis in the adult. We hypothesize than an alteration of the morphogen signalling microenvironment is the driver for the altered configuration of the tissue observed in pre-cancerous conditions of the gastrointestinal tract. The project's main objective is to use spatial transcriptomics to map the molecular triggers dictating morphological and cellular composition changes in the gastro-intestinal diseased mucosa. We will focus on the detection of morphogens and growth factors involved in cellular regeneration and differentiation, and we plan to harness our established mucosoid cultures, an evolution of organoids, to test the different growth factors combinations *in vitro*.

We use fixed samples of gastrointestinal biopsies from healthy individuals and from patients with pre-cancerous conditions or lesions. We are particularly interested in the etiogenesis of oesophageal, stomach and colorectal cancer. Three patients per condition will be analysed using the GeoMX whole genome spatial transcriptomic profiling provided by NanoString®. Transcriptomic data from this experiment are analysed to extract information about the expression of morphogenic signals, their receptors and downstream target genes. The activity of morphogenic pathways is tested on mucosoid cultures using synthetic morphogens and corresponding pathway inhibitors. Upon stimulation with morphogens, cells in the mucosoids cultures can regenerate and differentiate into the different stomach lineages as they do inside the organ.
Mapping growth factors directly in the original human tissue and testing their function on relevant human-derived cultures promises to be a robust strategy to understand mechanisms of carcinogenesis

Research Objectives
Aim1) Generating a spatial map of the morphogenic signals during gastrointestinal disease progression.
We will profile the transcriptome of different parallel regions of healthy and pre-cancerous gastrointestinal mucosa (eg: Barrett's oesophagus, intestinal metaplasia, colon polyps). Our clinical collaborator and co-supervisor Dr. Jan Bornschein is involved in the identification of the patients for this project. Prof Fadi Issa, runs Nanostring at the department of surgical science at University of Oxford. We will focus on the detection of genes related to morphogen signalling pathways involved in cellular regeneration and differentiation. The expression of these genes, receptors and related transcription factors will be mapped in the different region of interest of the epithelium, stroma and cell of the immune system. The comparative analysis of the transcriptomic profiles will be performed using the software "SignaLink3" [1] to identify the gene interactome and the putative pathways crosstalk. The software was developed by T. Korcsmaros, a collaborator in this project.

Aim II) Assessing the role of morphogens in driving epithelial differentiation, regeneration and proliferation.
The pre-cancerous conditions of the gastrointestinal mucosa are characterised by a different morphology but also by a disbalance in the cell population lineages. The morphogen signalling ligands identified in Aim 1 will be tested on mucosoid cultures originated from the same biopsies. Mucosoids are a patented [2] development of the organoid cultures; cells are cultivated in a monolayer forming an epithelial barrier which is very similar to the gastrointestinal epithelium [3, 4]. Cells within the mucosoids can differentiate upon stimulation [5]. By adding ligands or pathway inhibitors in the cultivation cocktail of the mucosoid cultures it is possible to determine their role in epithelial cell regeneration, proliferation and differentiation using different published functional or biochemical assays [3, 5]

Translational potential

Although there is a strong focus on understanding the microenvironment of cancer and the contribution of neighbouring non-transformed cells to the disease, little is known about the microenvironment of pre-cancerous conditions, and an unbiased approach to map all the morphogens has never been attempted. We aim to find dysregulations in specific morphogen signalling cascades that are predictive for disease progression. The gold standard for the detection pre-cancerous conditions is endoscopy and tissue imaging. Alternative serological analysis is accurate, but have a low sensitivity. A combination of ligands or proteins involved in morphogen signalling pathways could be use as surrogate of those conditions to develop diagnostic tests for pre-cancerous conditions and to predict risk of progression

Training opportunities

Day-to-day supervision and training will be provided by Francesco Boccellato and from post-docs in the lab. The student will have the opportunity to learn cutting edge technologies such as spatial-transcriptomic and organoid and mucosoid cultures. We expect the student to become proficient into data analysis and we will support this by encouraging the attendance to bioinformatic courses. Imaging with confocal microscopies and standard biochemical assays are also part of the basic training.

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12. ADP-ribosyl hydrolase as a biomarker for PARP inhibitor sensitivity/resistance [1,2,3,4] – Prof. Ivan Ahel

Primary Supervisor: Prof. Ivan Ahel
Additional Supervisors: Prof. Ahmed Ahmed
Eligibility: All tracks are eligible to apply for this project.

Abstract
To protect the genome from damage organisms have evolved a cellular defence mechanisms termed the DNA damage response (DDR). The DDR includes a diverse set of signal transduction pathways and effector proteins that act to sense DNA lesions and effectively repair the damage, limiting the propagation of genomic instability. Exploiting DDR pathways to specifically target and kill cancer cells has become an attractive therapeutic avenue within cancer research. This is exemplified by the synthetic lethal interaction between PARP inhibition and *BRCA1* or *BRCA2*-deficient tumours[1]. Ivan Ahel (co-supervisor on this project) laboratory recently identified HPF1 protein as a novel interactor and critical regulator of PARP1 ADP-ribosylation activity upon DNA damage[2]. Functionally, HPF1 suppresses DNA damage-induced hyper auto-modification of PARP1 and promotes *in trans* ADP-ribosylation of histones and many other proteins involved in regulation of genome stability. They further demonstrated that HPF1 is a critical specificity factor that allows modification of target proteins by PARP1 on serine residues (Ser-ADPr)[3,4]. Crucially, the work also identified ARH3 as a hydrolase which specifically removes Ser-ADPr[5] and further showed that Ser-ADPr is the major form of ADP-ribosylation following DNA damage[6]. Taken together, the insights surrounding Ser-ADPr open a large, exciting, and novel area of research into the fundamental understanding of the pathways regulated by this modification. Strikingly, our recent data show that ARH3 knockout in model cell lines associates with PARP inhibitor (PARPi) resistance, while ARH3 overexpression is associated with PARPi sensitivity[7]. Based on these results, we hypothesize that ARH3 activity and protein levels affect sensitivity to PARPi, thus representing; i) a predictor for the success of these therapies and, ii) a novel target for further drug development. Currently, PARP inhibitors are used to treat ovarian cancer and several other cancers, and we therefore propose to test the hypothesis that ARH3 expression might be a useful diagnostic tool with which to stratify cancer patients into sub-groups that will be sensitive/resistant to PARPi treatment with a particular focus on ovarian cancer. The mechanism of sensitivity/resistance of cells with deregulated ARH3 expression cells to PARPi is unknown, and elucidating this mechanism will be another goal of this proposed work.

Research objectives
Objective 1. Characterise the effect of ARH3 under- and overexpression in a series of model and primary cancer cell lines on PARP inhibitor sensitivity/resistance. We will collect and test a variety of ovarian cancer cell lines, profiling them for ARH3 protein expression levels and then treating with several different PARPi of varying PARP-trapping capabilities (olaparib, talazoparib, veliparib). To determine the impact of ARH3 protein levels on PARPi vulnerability, we will not only assess drug sensitivity and levels of PARP1, PARG, and ARH3 across a panel of ovarian cancer cell lines, but also assess the impact of systemically varying ARH3 by knockdown, knock out and inducible overexpression in HGSOC lines of defined genotype, including Ovcar8 (BRCA1/2 wt, PARPi resistant), PE01 (BRCA2-mutant, PARPi sensitive), Kuramochi (BRCA2-mutant, PARPi partially sensitive) and COV362 (BRCA1-mutant, PARPi sensitive). Rescue experiments with wild type vs. catalytically inactive ARH3 will assess the suitability of ARH3 as a target for the development of inhibitors.
Objective 2. To determine the frequency of ARH3 gene alterations in a larger set of HGSOC samples, we will: i) interrogate data of an ongoing whole exome sequencing study of 504 ovarian cancers searching for ARH3 and PARG copy number alterations and mutations; and ii) perform semi-quantitative detection of ARH3, as well as of PARG, PARP1 and PAR, by immunohistochemistry (IHC) on two independent sets of tissue microarrays (TMAs) containing a total of 1200 ovarian cancers. To augment these analyses, which will be limited by the small number of tumors treated with PARPi, we will also evaluate levels of ARH3, PARG, PARP1 and PAR in patient-derived xenograft (PDX) models that have been assayed for response to single-agent PARPi, including ones that have a high HRD score but did not respond. This objective will be performed in co-supervisor (Prof Ahmed Ahmed) laboratory at the Nuffield Department of Women's & Reproductive Health, University of Oxford.

Objective 3. Elucidating the mechanistic basis for the sensitivity/resistance of cells with deregulated ARH3 expression cells to PARPi (modulation of the PARP-trapping, regulation of DNA repair pathway choice, regulation of the chromatin structure/epigenetic marks). For these studies we will use largely cell biology/biochemical and genomics approaches.

Translational potential

Our data suggest that ARH3 protein expression levels in cancer patients might be a marker that confers sensitivity/resistance of the tumour to PARPi, providing a rationale for using PARPi for certain patients. In longer term, understanding the mechanisms of DNA repair and PARPi resistance through studies of ARH3 protein, may reveal new, unexpected avenues for treatments in the future.

Training opportunities

The student will have opportunities to train in diverse set of methods including cell biology/cell culture approaches for structure/function analyses, well-established cell survival assays that we be applicable for wide range of cell toxicity studies, immunohistochemistry methods and patient-derived xenograft (PDX) models.

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13. Improving Immunotherapeutic Efficacy in Colorectal Cancer Using Ultrasound-activated Nanoparticles and Image-guided Drug Delivery [1,2,3,4] – Prof. Tim Elliot

Primary Supervisor: Prof. Tim Elliot
Additional Supervisors: Associate Prof. Robert Carlisle
Eligibility: All tracks are eligible to apply for this project.

Abstract
Cancer immunotherapy using immune checkpoint inhibitors (ICI) targeting the programmed cell death-1 receptor (PD-1) and its ligand (PD-L1) are routinely used in clinic for treating solid tumours[1]. However, >95% of colorectal cancers are immunologically 'cold', microsatellite stable and DNA mismatch repair proficient tumours that do not currently benefit from immunotherapy[2]. Treatment failure is often due to factors e.g. low neoantigen burden, loss of MHC-I, dysfunctional antigen processing and presentation, lack of tumour-infiltrating lymphocytes and local immunosuppression. Furthermore, the presence of dense stroma and dysregulated tumour blood vessels can serve as physical and functional barriers to drug delivery. There is an unmet need for the development of therapeutic and image-guided approaches to enhance drug delivery into and throughout tumours and transform a 'cold' immune-excluded tumour into a 'hot' immune-inflamed tumour.

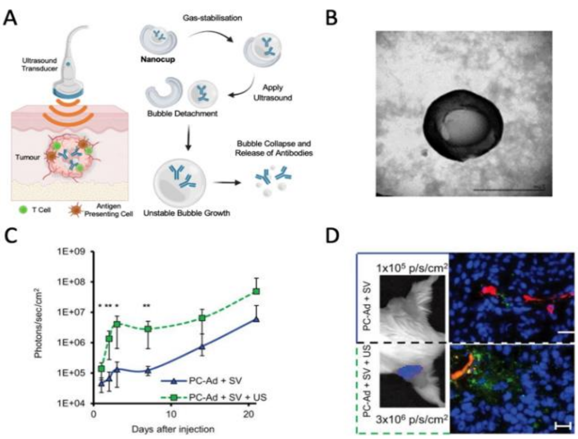

Figure 1. Ultrasound-activated nanoparticles for delivery of drugs.

(A) Schematic diagram for ultrasound-guided delivery of nanoparticle encapsulated treatment antibodies. (B) TEM of novel ultrasound-responsive solid particles manufactured using immunostimulatory proteins. (C) and (D) Improved delivery of oncolytic adenoviruses ('PC-Ad') using microbubbles ('SV') and ultrasound. Green (virus), red (blood vessel), blue (DAPI). Images were adapted from [5] and [7].

Focused ultrasound has emerged as a promising approach for immuno-modulation of tumours through thermal or mechanical perturbation[3]. Ultrasound-mediated cavitation using novel, biocompatible nanoparticles has been used to enhance the delivery of chemotherapy and oncolytic viruses into tumours[4,5] (Figure 1). Ultrasound itself is a relatively low-cost system used in clinical imaging of soft tissues, tissue stiffness and tumour blood flow, and can be used for real-time monitoring of drug delivery. The Elliott Group at the Centre for Immuno-Oncology investigates the mechanisms of antigen processing and presentation involved in T cell response to cancer and during immunotherapy using biochemical, computational, and physical science methods. The Biomedical Ultrasonics, Biotherapy and Biopharmaceuticals Laboratory (BUBBL) at the Institute of Biomedical Engineering specialises in the development of novel ultrasound-activated nanoparticles for image-guided drug delivery. This multidisciplinary project is a partnership between immuno-oncology and biomedical engineering.

Research objectives
The aim of this project is to develop and validate ultrasound-activated microbubbles (MB) and solid sonosensitive particles (SP) to actively transport and enhance the distribution of ICI in tumours via mechanical cavitation. The treatment efficacy of ultrasound-activated cavitation agents as propellants or vehicles for ICI delivery and the mechanistic effects of cavitation on antigen presentation, T cell avidity and function will be investigated. Preclinical colorectal cancer models already comprehensively characterised by the Elliot group will be used[6,7].

***Work Package 1*: Development of ultrasound-activated nanoparticles for targeted drug delivery**
PD-1 and/or PD-L1 monoclonal antibodies of different doses will be co-delivered with SP or formulated into SP or MB. The physicochemical properties of the SP and MB formulations, doses of PD-1/PD-L1 monoclonal antibodies delivered with, or formulated into, each cavitation agent and the acoustic parameters for tumour delivery will be optimised, and correlated to the tumour volume, microvessel density and stromal thickness. For microscopic evaluation of the drug spatial distribution, PD-1 and/or PD-L1 monoclonal antibodies will be bioconjugated with amine-reactive reagents for fluorescent labelling with near infrared dyes. *WP1 will be performed in collaboration with Prof. Constantin Coussios and Prof. Eleanor Stride.*
***Work Package 2*: Establishment of a preclinical colorectal cancer model for novel treatment and mechanistic studies**

The BALB/c mouse model of colorectal carcinoma, CT26 is a microsatellite stable and DNA mismatch repair proficient tumour which shares molecular features with aggressive, undifferentiated, and refractory human colorectal cancer. CT26 is one of the most extensively investigated syngeneic tumour model in preclinical studies and has been used to validate most immunotherapeutics currently in the clinic or under clinical trials, with >500 studies in literature. SP and MB formulations with optimised PD-1 or PD-L1 doses will be administered into mice bearing subcutaneous CT26 tumours and actively delivered into the tumours via ultrasound-mediated cavitation. Tumour growth monitoring will be conducted to determine treatment efficacy and response classification into progressors or regressors. Tumours and secondary lymphoid organs will be harvested at the study endpoint for functional analyses using multiparametric flow cytometry, transcriptomics, immunofluorescence staining and multiplex imaging. In particular, biomarkers related to MHC Class I antigen presentation, antigen processing e.g. tapasin and calreticulin, $CD8^+$ and $CD4^+$ T cell, antigen-presenting cells such as dendritic cells, immunosuppressive cells, cancer-associated fibroblasts, extracellular matrix remodelling, vascular inflammation and tumour hypoxia will be examined closely. Experiments will be designed to distinguish the immunological effects of cavitation alone from the effects of enhanced antibody delivery instigated by cavitation. *WP2 will be conducted in collaboration with Dr. Doreen Lau and A/Prof. Joanna Hester.*

***Work Package 3**: Understanding the effects of novel treatment on antigen presentation and T cell dynamics in cancer*

The mechanical and immuno-modulatory effects of the optimised nanoparticle formulation and focused ultrasound on antigen presentation and T cell dynamics in cancer will be examined with immuno-profiling, biophysical measurements and live imaging. Tumour antigen-specific $CD8^+$ T cells of high versus low functional avidity will be identified based on pMHC tetramer staining and image-based biophysical measurements of overall pMHC:TCR binding strength using acoustic force spectroscopy. These T cell populations will be fluorescently labelled and adoptively transferred into CT26 mice prior to treatment with this novel approach. Non-invasive imaging of tumour stiffness using ultrasound elastography and vascular permeability and perfusion using contrast-enhanced ultrasound will be conducted before and after treatment to derive clinically relevant imaging biomarkers of stromal and vascular response to treatment. Imaging at the single-cell level will be performed using two-photon microscopy to examine the migration and cellular kinetics of tumour-specific T cells across physical barriers (tumour stroma and vasculature). This will be conducted on vibratome-sliced tumours in perfusion chambers with dye-labelling of the tumour blood vessels for visualisation and second harmonic generation imaging of the stromal collagen fibres at the tumour invasive margin. Image processing and analysis of the fluorescent T cell tracks, velocities, and spatial confinement within different tumour compartments (peritumoral versus intratumoral), T cell spatial distribution and distance to tumour cells, tumour stroma and blood vessels will be conducted[8]. *WP3 will be done in collaboration with Dr. Doreen Lau and Prof. Eleanor Stride.*

Translational potential

This work will develop and validate novel ultrasound-activated nanoparticles for image-guided delivery of ICI in preclinical colorectal cancer models. The aim is to investigate whether drug penetration can be optimised using this approach and better understand its mechanistic effects on antigen presentation, T cell avidity and function in tumours. An Oxford spin-out company, OxSonics Therapeutics, in which a similar cavitation technology (‘Nanocups’) is based, has already reached clinical-stage and is currently in Phase I/II clinical trial for ultrasound-guided delivery of anti-cancer agents in metastatic colorectal cancer patients. The difference in the present proposal is the introduction of a sonosensitive particle that is capable of not only enhancing antibody delivery and distribution, but also in promoting the local immune response through the introduction of immuno-stimulatory proteins adjacent to the cavitation process. A long-term plan for this project would be to translate the technology and discoveries into clinics to optimise the efficacy of immunotherapeutics.

Training opportunities

This project is ideally suited for non-clinical candidates with a background in pharmacology, biomedical sciences or bioengineering and is also suitable for clinical candidates wishing to gain research experience in preclinical immunotherapy modelling and novel drug delivery in an interdisciplinary setting. Training on chemical engineering, tumour immunology, cellular imaging with two-photon microscopy and acoustic force spectroscopy, as well as the use of therapeutic and **diagnostic** imaging ultrasound will be provided. More general research, communication, teaching, innovation, and career development skills training will be given by the Medical Sciences Division.

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14. Uncovering the regulation and functions of supermeres in colorectal cancer - [1,2,3,4] – Prof. Clive Wilson

Primary Supervisor: Prof. Clive Wilson
Additional Supervisors: Prof. Adrian Harris and Prof. Chris Cunningham
Eligibility: All tracks are eligible to apply for this project.

Abstract
Defective communication between cells is a key factor in cancer formation and progression. Traditionally, such signals are considered to involve single molecules like growth factors, but recently, more sophisticated multimolecular assemblies, such as extracellular vesicles (EVs) and non-vesicular nanoparticles, which can harbour a wide range of activities, have emerged as alternative mediators. Supermeres are a new type of protein:RNA complex produced by many cancer cell types[1]. They are enriched with cargos that are upregulated in a wide range of cancers, for example, glycolytic enzymes, TGFBI, miR-1246, MET, GPC1 and AGO2. Most extracellular RNA is reported to be associated with supermeres and not EVs. Cancer-derived supermeres increase lactate secretion, transfer cetuximab resistance and reduce hepatic lipids and glycogen *in vivo*. However, the cellular origin of supermeres and their links with other secreted multimolecular complexes are unknown, hindering their detailed analysis. We previously used a *Drosophila* prostate-like cell with highly enlarged endosomal compartments to unpick the biology of exosomes, small EVs generated in these compartments. This work revealed a previously unidentified, evolutionarily conserved exosome subtype called Rab11a-exosomes made in recycling endosomes, which appear to control cancer progression[2]. Although they represent only a small fraction of secreted EVs, they are major mediators of several EV-associated functions[3]. We have now identified protein aggregates in our fly system with a similar protein signature to human supermeres, shown that their biogenesis is interlinked with Rab11a-exosome formation, and identified genetic manipulations of highly conserved molecules that block their assembly[3-6] (and see below). We hypothesise that human supermeres are generated in recycling endosomes via similar mechanisms to those in the fly and that these compartments play a central role in cancer cell communication. In this project, the student will test this idea in colorectal cancer (CRC) cells, where we first identified Rab11a-exosomes. Ongoing molecularly stratified trials and collection of serial blood samples from patients will allow the specific functions of supermeres and their potential as biomarkers to be tested. The student will spearhead the analysis of this new form of cancer cell signalling, working with basic scientists and clinicians who have diverse skills and expertise.

Research objectives
The project has four research objectives and proposed outcomes:

2.1 Determine the role of cellular stress responses in supermere release: In our fly model, supermere-like structures coalesce in Rab11a-positive recycling endosomes to form very large central dense-core granules[3-6]. Like human supermeres[1], TGFβ-induced (TGFBI, a highly conserved secreted molecule) is the most abundant protein in these complexes (Fig. 1A), which are also enriched in other supermere cargos, such as GPI-anchored proteins, proteases[6] and cleaved membrane proteins, like Amyloid Precursor Protein (APP). Our studies reveal that complex formation is dependent on TGFBI (Fig. 1) and Rab11a-exosomes[3-5]. In recent unpublished findings, we have shown that other oncogenic proteins control this process, and that APP-like proteins play a key role in separating Rab11a-exosomes from these aggregates. This separation regulates the activities of both the exosomes and the aggregates.

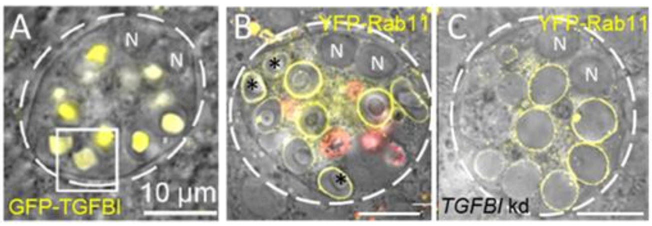

Fig. 1. TGFBI is a key regulator of protein aggregation in flies. A. GFP fusion to *Drosophila* TGFBI expressed from the endogenous gene is expressed specifically in dense-core granules (yellow circles) in large compartments (one outlined by square) in a specific secretory cell (outlined with dashed ellipse). **B.** Compartments containing these cores (some labelled with *) are marked by recycling endosomal Rab11. **C.** *TGFBI* kd blocks aggregation, but compartments retain Rab11 identity. N = nuclei. Scale bar = 10 µm.

To determine whether human supermeres are selectively produced in Rab11a-compartments, we will test whether supermere release is affected in CRC cell lines, eg. HCT116, SW620, by stresses that promote Rab11a-exosome secretion, eg. nutrient depletion, hypoxia, drugs that block growth factor signalling. Supermeres will be isolated by ultracentrifugation; if they are abundant in non-stressed, as well as stressed, cells, their composition will be assessed by western/miRNA analysis, to determine whether, like exosomes, their structure is altered by their origin.

Proposed outcome: Determine the origin and composition of supermeres generated under different stress conditions.

2.2 Identify regulators of supermere biogenesis: We will genetically manipulate CRC cells under both nutrient-depleted and -replete conditions, knocking down genes involved in the formation of supermere-like structures in flies, eg. *TGFBI*, *ESCRT*s (to suppress exosome biogenesis), etc, and test how supermere release is affected. Our current data suggest that *TGFBI* knockdown (kd) will selectively block supermere formation and this will change the activities of the cancer cell secretome; the resulting preparations, depleted of supermeres, will be analysed by proteomics and transcriptomics, to more fully determine, through a process of elimination, the composition of these structures.

Proposed outcome: Define mechanisms controlling supermere generation in CRC cells and identify specific supermere cargos.

2.3 Determine cancer-related functions of supermeres: Cancer supermeres are readily internalised within target cells and have multiple functions in metabolism and drug resistance[1]. We will confirm this using supermere preparations from nutrient-depleted and -replete cells to determine whether supermere signalling changes under stress. We will also test the functions of conditioned medium from cells +/- nutrient-depletion, +/- kd (eg. *TGFBI* kd) that blocks supermere release to assess supermere function in the presence of other signals, eg. exosomes. We will include assays for metabolic changes[1], drug resistance[1], including oxaliplatin and 5FU, and effects on endothelial[2] and other stromal cells.

Proposed outcome: The functions of supermeres in the presence or absence of other signals will be defined.

2.4 Assess the therapeutic implications of supermeres in CRC patients: Recent unpublished studies indicate that some Rab11a-exosome markers from CRC cell lines are detected in EV preparations from CRC patients' plasma and these may be differentially expressed in patients that respond differently to neoadjuvant chemoradiotherapy (nCRT). We will isolate supermeres from plasma of these and other patients, both pre- and post-therapy, and from blood-bank controls, confirming integrity after freeze-thaw, testing effects of different anticoagulants, etc; protein and miRNA content will be assessed in relation to therapeutic response.

Proposed outcome: These preliminary studies will assess whether supermeres carry biomarkers that relate to CRC or response to nCRT, and pave the way for more patient-focused studies to block supermere function in the future.

2.5 Academic value of research: Signalling by multimolecular complexes is emerging as a key, but poorly understood, mechanism by which normal and cancer cells can completely reprogramme target cells and their microenvironment. Wilson's lab has made a number of fundamental science discoveries that have opened up new ideas and approaches that allow the analysis for these processes. The group has a strong track record in discovery science with relevance to cancer biology[2-7], then developing ideas translationally through collaborations (see ***2.6***). The student will play a central role in taking a similar approach to characterise cancer supermeres.

2.6 Collaborations involved and how these will be facilitated by the award: Profs Adrian Harris and Clive Wilson, working with Assoc. Prof Deborah Goberdhan have a strong, long-standing track record of collaboration in tumour cell biology, in which *Drosophila* has informed cancer studies, eg [2,8]. Prof Chris Cunningham has collaborated with Goberdhan and Harris to study exosomes in CRC patients. This project brings these teams together to work in a completely new area, supermere biology, so that fundamental discoveries in flies can again be exploited in clinically related studies. The project will facilitate these interactions and increase the opportunities for subsequent translation.

Translational potential

There is increasing evidence that multimolecular complexes, like supermeres, play central roles in cancer signalling, but defining those roles and assessing the translational potential requires advanced cell biological and genetic analysis. Our fly studies have opened up the opportunity to gauge the importance of supermere signalling in CRC and other cancers and determine the potential relevance of supermeres to early detection, patient stratification, prognosis and therapy, guided by the expertise of the collaborating supervisors (see ***2.6*** above). This project will provide the proof-of-principle work for future patient-focused, CRUK-funded studies in this area.

Training opportunities

The student will use a range of cancer-relevant techniques, including advanced cell culture and biochemical methods, cell biology, molecular genetics, high-resolution fluorescence imaging and bioinformatics. They will also develop expertise in cancer signalling, working at the interface of the collaborative environment we have established. Overall, this work should open up a new field in cancer biology, relevant to CRC and other cancers.

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15. Improving CAR-T cells for B-ALL[1,2,3,4] – Prof. Omer Dushek

Primary Supervisor: Prof. Omer Dushek
Additional Supervisors: Prof. Ronjon Chakraverty
Eligibility: All tracks are eligible to apply for this project.

Abstract
T cells patrol the body in search of antigens derived from infectious organisms or cancer cells. They use their T cell antigen receptors (TCRs) to recognise peptide antigens on major-histocompatibility-complexes (pMHC). T cells have remarkable antigen sensitivity; they can become activated when recognising only a single pMHC. This high sensitivity is important because infectious organisms and cancers deploy evasion mechanisms to reduce the amount of antigen presented to T cells. T cells are now engineered to recognise cancer antigens using chimeric antigen receptors (CARs). This therapy is approved to treat B cell cancers. However, many patients relapse with B cells that express low levels of the target antigen. It is now clear that CARs have a profound defect in antigen sensitivity; CARs require 100-1000-fold more antigen than the TCR to activate T cells. There is an urgent need to increase the sensitivity of CARs to prevent these relapses. The high antigen sensitivity of the TCR is partly a result of their adhesion receptor CD2 binding to its ligand CD58. In recent work, we have found that CARs fail to efficiently exploit the CD2/CD58 adhesion interaction. Here, we will use our basic understanding of how CD2 functions to generate novel CARs that efficiently exploit CD2 and test them using *in vivo* relapse models of B cell acute lymphoblastic leukaemia (B-ALL). This will improve existing CAR-T cell treatments for B cell cancers and should allow for new treatments to eliminate cancers expressing low levels of target antigens.

Background. The T cell antigen receptor (TCR) has remarkable antigen sensitivity; it is able to recognise even a single peptide antigen on target cells (1). It achieves this high sensitivity in part by exploiting the T cell adhesion receptor CD2 binding to its ligand CD58. Recent studies have shown the CD2/CD58 interaction to play critical roles in T cell function, including recognition of cancerous and infected cells (1).
Adoptive cell transfer (ACT) of genetically engineered T cells expressing Chimeric Antigen Receptors (CARs) is a clinically approved cancer therapy for haematological malignancies (2). CARs are synthetic receptors that are generated by the fusion of an antibody-derived, antigen-binding single-chain variable fragment (scFv) with intracellular signalling motifs from the cytoplasmic tails of the TCR complex. Although CAR-T cells targeting the B cell surface antigens such as CD19 are initially highly effective, a large fraction of patients relapse when malignant cells emerge with reduced expression the target antigens (2). It is now appreciated that CARs are relatively insensitive, requiring 100 to 1000-fold higher antigen densities to induce T cell activation compared to the native TCR (2). However, the mechanism underlying their defect in antigen sensitivity is presently unknown. There is now an urgent need to improve the antigen sensitivity of CAR-T cells in order to treat patients with relapse and to avoid relapse in the first place.

Research objectives
In recently completed work, we have identified that the mechanism underlying the antigen sensitivity of CARs is their inability to efficiently exploit the CD2/CD58 interaction (3). We found that CD2 increased the antigen sensitivity of the TCR by 125-fold but only <5-fold for CARs. Here, we will use our basic understanding of how CD2 improves antigen sensitivity of the TCR to produce novel synthetic antigen receptors that efficiently exploit CD2.

Objective1. Produce a highly sensitive synthetic antigen receptor targeting CD19.

Objective 2. Use pre-clinical models of B-ALL relapse to benchmark the *in vivo* activity of our novel synthetic receptor (see Objective 1).

Objective 2. Use *in vivo* findings (Objective 2) to inform on improved design (Objective 1).

The Dushek and van der Merwe laboratories have a long-standing history of studying antigen recognition by the T cell receptor, including understanding the role of accessory receptors such as CD2. Recently, they have developed an experimental system to analyse the antigen sensitivity of CARs relative to the TCR. They will lead on producing highly sensitive CARs targeting CD19 (Objective 1). The Chakraverty laboratory is focused on using murine models to understand the mechanisms of relapse to approved CD19 CARs. They will lead on *in vivo* testing (Objective 2) and have recently establish a model in their laboratory based on a previous report (4). There are now examples where the surface expression of CD19 is not only reduced but is completely abolished. If synthetic antigen receptors with higher sensitivity are insufficient to avoid this cancer evolution process, the study of relapse can identify preserved surface proteins that can be targeted at the outset alongside CD19 (Objective 3).

Translational potential

Currently, CARs underlie approved cancer treatments and a large number of promising treatments (e.g. using not only T cells but NK cells and macrophages). It follows that fundamental improvements to their antigen sensitivity can immediately be adopted by the large community of academic and industrial researchers.

Training opportunities

T cell isolation, tissue culture, synthetic design and genetic modification (CRISPR, Lenti-transductions), flow cytometry, mathematical modelling (to understand mechanism), pre-clinical models of B cell cancers.

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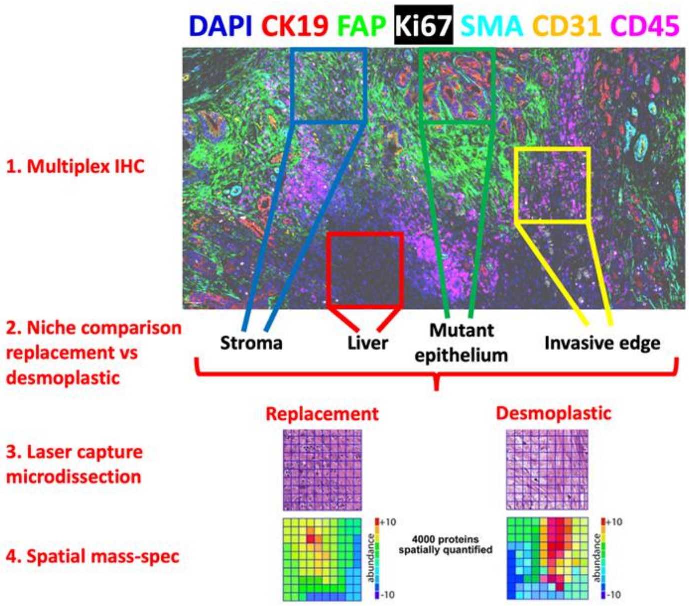
16. Deciphering spatial differences in Histopathological subtype of colorectal cancer liver metastasis (CRLM) [1,2,3,4] – Dr. Alex Gordon-Weeks

Primary Supervisor: Dr. Alex Gordon-Weeks
Additional Supervisors: Prof. Simon Buczacki
Eligibility: All tracks are eligible to apply for this project.

Abstract
CRLM occur in 50% of colorectal cancer patients and metastasis is the most common cause of colorectal cancer death. Surgical resection is considered the most successful treatment option, but recurrence occurs in up to 75% of patients within 5 years of attempted curative surgery. However, the outcomes following surgery are highly heterogenous. The strongest predictor of good versus poor outcome is the histological growth pattern. CRLM grow either in a replacement pattern (cancer cells at the invasive edge invade the hepatic parenchyma, co-opting hepatic vasculature) or a desmoplastic pattern (the invasive edge is characterised by a rim of inflammatory tissue consisting of fibroblasts and immune cells). The



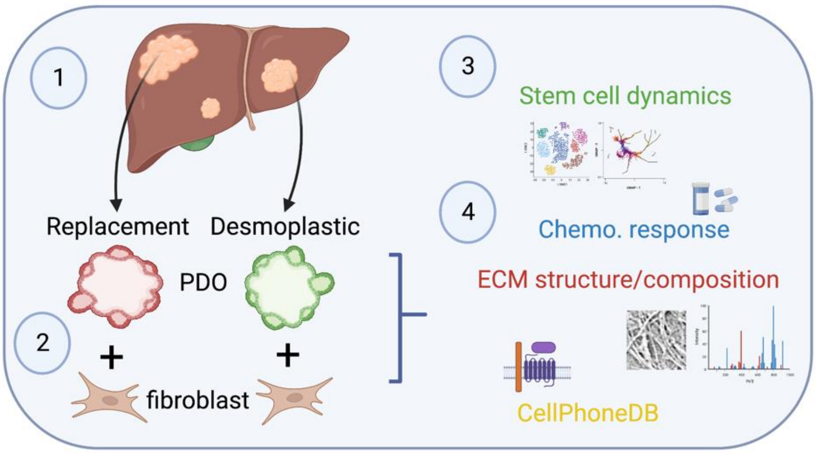


Figure 1.

Through ***multiplexed immunohistochemistry*** (GE Cell-Dive) of CRLM from replacement and desmoplastic morphologies, biologically distinct niches are defined. Niche cellular composition is compared between growth patterns to identify differences in cellular ecology. Niches of interest (those with significantly different cellular composition between growth patterns) are chosen for ***spatial proteomics*** to interrogate differences in cell-cell communication networks and deposited ECMs within the niches. This approach will provide an enhanced understanding of the biological differences between the growth patterns identifying potential therapeutic targets.

Co-culture experiments combining ***CRLM patient-derived organoids*** (PDOs) of known histological subtype with primary liver fibroblasts will enable us to interrogate the cellular interactions that provide a basis for the multiplexed IHC and proteomics observations. Downstream analysis will include assessment of PDO morphology and stem cell dynamics through single-cell RNA-seq and the growth pattern-specific fibroblast response through analysis of fibroblast-derived extracellular matrix. Here, pharmacological methods to switch plastically between pathological subtype will be explored.

replacement subtype has a significantly worse overall survival when compared with the desmoplastic subtype, but very little is known about the biological drivers linking histological growth pattern to outcome.

Research objectives

1. Perform mIHC and downstream image analysis on CRLM samples from patients with replacement and desmoplastic tumours.
2. Perform spatial proteomics on identified niches of interest within CRLM specimens and work with bioinformaticians within Professor Roman Fischer's group to analyse the data outputs.
3. Identify replacement-dependent signalling mechanisms by comparing PDO-fibroblast co-cultures to identify tractable therapeutic targets for interchanging between histological subtypes.

Outcomes

1. Gain a completely novel understanding of the CRLM cellular and proteomic microenvironment and understand for the first time how this differs between CRLM histological subtypes.
2. Identify mechanisms through which histopathological subtype can be manipulated, providing a potential patient-tailored therapeutic solution.

Training opportunities

1. Patient-derived organoid isolation, organotypic culture, CRISPR-Cas9 gene editing, single cell sequencing of organoids and extracellular matrix biology ***(Alex Gordon-Weeks/Simon Buczacki)***
2. Mass spectrometry, proteomics, metabolomics, data analytics/integration ***(Roman Fisher)***[1,2]
3. Multiplexed IHC performance and image analysis ***(Mark Coles)***

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17. Targeting innate immunity for intestinal injury recovery - [1,2,3] Dr. Monica Olcina

Primary Supervisor: Dr. Monica Olcina
Additional Supervisors: Prof. Simon Buczacki
Eligibility: Track1, 2, and 3 students are eligible to apply for this project.

Abstract
Effective radiotherapy requires a suitable therapeutic window allowing appropriate tumour control without excessive toxicity to healthy tissues. Further improvements in our understanding of the mechanisms underlying treatment-induced normal tissue injury may lead to the identification of therapeutic targets that reduce normal tissue toxicity (ideally while simultaneously improving tumour response). We are interested in understanding the role of druggable innate immunity proteins that, when targeted, simultaneously improve tumour response and reduce normal tissue toxicity. Our previous work has identified complement receptor, C5aR1 as a key modulator of both tumour response and radiation-induced bowel toxicity [12]. While C5aR1 is well-known for its role in the immune compartment, we find that C5aR1 is also robustly expressed on malignant epithelial cells, highlighting potential tumour-cell specific functions. We identify that C5aR1 primarily regulates cell fate in malignant cells, and that C5aR1 targeting results in increased NF-kB-dependent apoptosis specifically in tumours and not normal tissues. Crucially, targeting C5aR1 improves tumour response while reducing normal tissue toxicity following irradiation in the abdominal cavity (**Figure 1)**

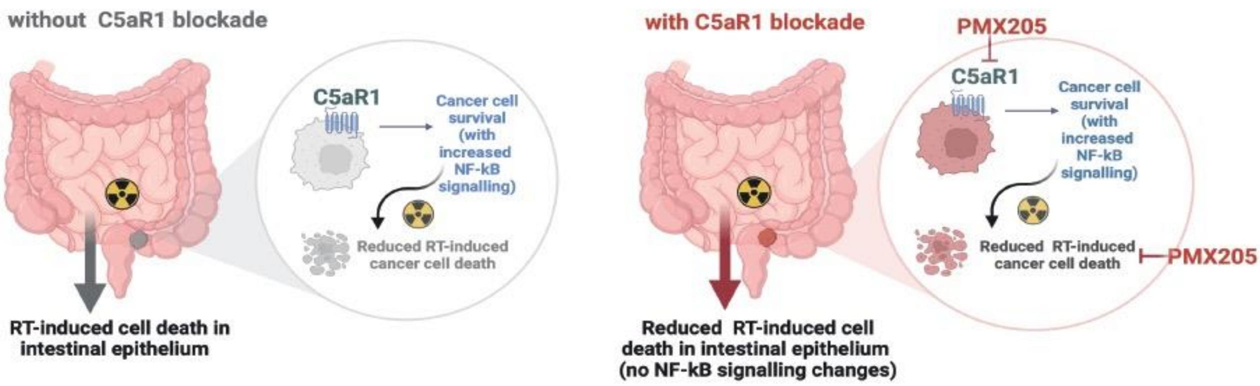

Figure 1. Working model for the effects of C5aR1 and C5aR1 blockade on tumour and normal tissue radiation (RT) responses.

The aim of this project is to investigate the role of C5aR1 in modulating recovery from bowel injury, including the mechanisms underlying the intriguing opposite effect of C5a/C5aR1 signalling in regulating cell fate in transformed and untransformed cells. Alongside these mechanistic studies we propose collecting patient samples to identify potential predictive markers correlating with normal tissue toxicity (and treatment response). There is increasing interest in investigating complement products as potential biomarkers, especially given that as soluble factors they can be easily detected from patient plasma. We hypothesize that C5a/C5aR1 signalling is key to recovery from normal tissue injury as well as tumour response to cytotoxic treatment such as radiotherapy. Therefore, levels of C5a could be used to predict predisposition to treatment/associated toxicity. Using patient plasma samples, we will explore whether C5a, as well as a range of other markers, are correlated with acute normal tissue toxicity and tumour response in plasma patient samples collected in collaboration with Dr Muirhead (Consultant Clinical Oncologist, Oxford University Hospitals).

Research objectives
The overall objectives of this project are to: 1) investigate mechanisms underlying recovery following treatment-induced intestinal injury; 2) Identify potential plasma markers correlating with susceptibility to treatment-induced injury.

Mechanistically, the student will investigate whether C5a/C5aR1 signalling regulates stem cell regeneration/differentiation either directly or indirectly through modulation of an inflammatory milieu. Understanding how C5aR1 signalling regulates intestinal injury will help us understand how to apply future C5a/C5aR1 targeting therapies to reduce treatment-related side effects.

Tools employed in this part of the project will include intestinal organoid culture (expertise available in the Buczacki lab). The student will also analyse spatial transcriptomics datasets from intestinal crypts of WT and C5aR1$^{-/-}$ mice (generated by the Olcina lab). Any changes observed will be validated using techniques such as qPCR, immunohistochemistry and functional assays (including in in vivo models). The student will be based across both groups to enhance collaborative working.

To identify potential markers of response, we will initially focus on two patient groups: 1) patients receiving stardard of care chemoradiotherapy for the treatment of primary tumours in the pelvic region. 2) Patients receiving SABR to different sites for the treatment of primary or oligomestatatic disease (in the context of continued immune checkpoint treatment). This will allow us to investigate markers of toxicity at a variety of sites and in the context of combined immune checkpoint and radiotherapy treatments. Additional samples from patients undergoing other treatments could be considered in the future.

The Olcina lab has expertise in collecting and processing patient plasma samples. The collaboration with the Buczacki lab will be greatly facilitated by having a joint student pursuing this project. Having a co-supervisor with expertise in intestinal stem cell biology will be key to the success of this project. The collaboration with Dr Muirhead will be critical to allow streamline patient recruitment and sample collection.

Translational potential of the project
Widening the therapeutic window of cancer treatments through normal tissue protection would be a critical step in treatment dose escalation, potentially increasing the chances of tumour control and improved patient quality of life. For example, diarrhoea induced following irradiation delivered for the treatment of tumours in the pelvic region is a dose-limiting toxicity for some abdominal tumours. This study will investigate the mechanisms underlying the intriguing opposite effect of C5a/C5aR1 signalling in regulating cell fate in transformed and untransformed cells. Such mechanistic detail will be critical for the most effective use of future C5aR1 inhibitors to increase the therapeutic window of standard-of-care cancer treatments. This work also has the potential to uncover biomarkers of normal tissue injury which could indicate which patients are at higher risks of toxicity. While the project will build on our previous work focusing on radiotherapy we will investigate whether these mechanisms also apply in the context of other treatments, including immunotherapy.

Training Opportunities
By undertaking this project, the student will benefit from working in two laboratories with complementary expertise in innate immunity and radiobiology (Olcina) and intestinal stem cell biology (Buczacki). Furthermore, the student will have the opportunity to develop expertise in patient sample handling, collection and processing in addition to a range of laboratory techniques including how to work with organoid cultures, flow cytometry, immunohistochemistry and standard cell and molecular biology techniques. The student will also have access to spatial transcriptomics data and will receive training in how to analyse these data. The student will likely also acquire experience in using colorectal cancer models with clinically relevant molecular phenotypes while working both independently as well as in a team. Career development opportunities will be offered, including attendance of national and international conferences and transferable skills training courses.

The collaboration with Dr Muirhead will provide an opportunity for the student to spend time in a clinical setting with the wider radiotherapy multidisciplinary team as well as patients to learn about clinical oncology, all facilitating a truly translational aspect to the project.

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18. Investigating an interplay between autoimmunity and cancer [1,2,3] – Prof. Xin Lu

Primary Supervisor: Prof. Xin Lu
Additional Supervisors: Prof. Lynn Dustin
Eligibility: Track 1,2 and 3 students are eligible to apply for this project.

Abstract

Reinvigoration of host immune systems to eliminate tumours is one of the most exciting developments in cancer therapy. Despite of its potential to achieve durable responses, only small percentage of patients respond to immunochemotherapy. A key challenge in immune-oncology field is to understand why some patients benefited from the treatment whereas others failed. Oesophageal cancer is the 6th most common cancer and incidence is rising rapidly and mortality is close to incidence. A major clinical challenge is to develop novel and effective therapies for oesophageal cancer. In 2021 FDA approved checkpoint blockade-based immunotherapy to treat inoperable oesophageal adenocarcinomas in combination with chemotherapy. It is therefore timely to identify molecular mechanisms that confer tumour sensitivity or resistance to immunochemotherapy.

To address this challenge, we will take the full advantage of our recently reported Lud2015-005 trial of oesophageal adenocarcinoma patients treated with anti-PDL1 alone for four weeks and followed with immunochemotherapy (Carroll et al, **Cancer Cell**, July, 2023). It is increasingly clear that tumour-associated B cells and their antibody products contribute to improved clinical outcomes and successful immunotherapy in diverse tumours. Antibodies may target normal self-antigens, tumour neoantigens, oncogenic viral proteins, and even endogenous retroviral gene products (Ng et al, Nature, April 2023). In many cases, autoantibody levels correlate with clinical benefit in patients treated with checkpoint blockade-based immunotherapy (CBI) either alone or in combination with chemotherapy or radiotherapy. Notably, patients in the Lud2015 trial with clinical benefit also tend to produce increased numbers of autoantibodies. We have cloned hundreds of autoantibodies from a number of Lud2015-005 trial patients with known clinical outcomes. This project will build on our recently published results and preliminary findings. The objective of this proposal is to test whether the observed increased autoantibody production, a common feature of autoimmune diseases, is simply a side effect of increased immune activity, or if the autoantibodies produced by tumour-associated B cells may contribute to cancer cell killing including antibody dependent cell-mediated cytotoxicity (ADCC). In this project, we will use a combination of cutting-edge molecular assays, organoids, co-culture techniques and single-cell sequencing. This work will form an essential component of our overall aim to improve future treatment of gastrointestinal cancers and inform the broader implementation of immunotherapy.

Research objectives

Background: Reinvigoration of host immune systems to eliminate tumours is one of the most exciting developments in cancer therapy. Therapies are being developed to inhibit pathways that tumours use to evade immune surveillance. Antagonists of the CTLA-4 and PD-1/PD-L1 immune checkpoint pathways (i.e. antibodies to CTLA-4, PD-1 or PD-L1) unleash previously suppressed T-cells to eliminate tumour cells. This strategy - termed immune checkpoint targeting therapy (ICT) - has achieved durable overall survival in patients with highly metastatic tumours. However, only a subset of tumours responds to ICT and understanding why many are resistant to ICT and ICT-related combination therapies is a major scientific challenge. Examining cellular responses before and after interventions is the key to address this problem. In 2012 it caused ~400,000 deaths worldwide and incidence is rising rapidly. Mortality has remained closely related to oesophageal cancer incidence, with a 5 year survival of <15%, indicating that many oesophageal cancer cells are resistant to existing therapies.

Objectives: The overall aim is to identify molecular differences before and after immune checkpoint therapy and between responders and non-responders. The objective of this proposal is to test whether the observed increased autoantibody production, a common feature of autoimmune diseases, is simply a read out of increased immune response or the autoantibodies produced by tumour associating B cells may positively contribute to cancer cell killing including antibody dependent cell-mediated cytotoxicity (ADCC).

Approaches: Key approaches include co-culture techniques, antibody-antigen interaction, organoid technology to explore interactions between tumour cells and immune cells. Single-cell sequencing may be used to dissect the cellular-level response to altered interactions. Additionally, autoantibody cloning, antibody-antigen interaction and antibody dependent cell-mediated cytotoxicity (ADCC) assays will be performed

Translational potential
This project is poised to have major implications for guiding future clinical decision making for patients with oesophageal cancer. Specifically, the connections made by the student between molecular characteristics and responses to therapy in the trial of immunotherapy in oesophageal cancer will be vital for developing new clinical stratification models.

Training opportunities
The overall strategy for the project and the laboratory research will be supervised by Prof. Xin Lu, Director of the Ludwig Institute for Cancer Research in Oxford, who has extensive experience of mentoring clinical and non-clinical DPhil students. There are opportunities to tailor the exact direction of this project to the interests and background of the trainee, with a focus on important emerging tools such as organoids, co-culture, single cell sequencing, TAPs sequencing technology to detect DNA methelome of cfDNA and autoantibody detection in liquid biopsies. Prof Lynn Dustin also has extensive mentoring experience and will provide expertise in autoimmunity. This project will enable the student to benefit from expertise and technologies at both the Ludwig Institute for Cancer Research and Kennedy Institute. The student will have opportunities to integrate with the wider scientific and clinical communities in Oxford through established collaborative networks, and with the national and international communities at conferences. The student will benefit from the training and career development programme at the Ludwig, which includes: regular oral presentations, journal clubs, and skill development in writing, data management and public engagement.

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19. An integrated systems biology approach to investigate the spatial Myeloma tumour microenvironment [1,2,3,4] – Prof. Udo Oppermann

Primary Supervisor: Prof. Udo Oppermann
Additional Supervisors: Assoc Prof. Adam Cribbs
Eligibility: All tracks are eligible to apply for this project.

Abstract
Multiple Myeloma (MM) is a plasma cell derived malignancy with the tumour residing within the bone marrow environment. Work on solid tumours provides clear evidence that the complex interactions of the cells constituting the tumour microenvironment play a pivotal role in mediating survival, proliferation, drug resistance, and progression of the cancer by shaping adaptive and innate immunity [1,2]. In regard to MM, the immune responses to therapy have been investigated using flow cytometry or single-cell NGS based platforms. Here, significant progress has been obtained to understand the immune composition and activation states of bone marrow aspirate-derived myeloma samples. However, a drawback of this approach has been the lack of spatial information that can be obtained. In order to fill this evident gap in understanding myeloma and its microenvironment the DPhil candidate will apply a novel and systematic approach that integrates multiple layers of information (i.e. targeted proteomics, transcriptomics, genomics) within a spatial context. This would significantly advance our understanding of myeloma phenotypes and interactions with immune and stromal cells and will help to better understand responses to therapy. We will use MM trephine biopsy samples obtained from routine clinical diagnostics and clinical trials and apply 'omics' approaches to these tissue sections. Following experimental data collection, the analysis will include the application of machine learning algorithms and will result in an integrated view of the myeloma tumour and its microenvironment.

Research objectives

Suggested overall workflow and clinical samples: In the first phase of the DPhil project (over the first 12-18 months) the candidate will acquire necessary basic skills such as sectioning of formalin-fixed paraffin embedded (FFPE) trephine biopsies, laser-capture microscopy, proteomics, multiplexed imaging, next-generation sequencing using in-house and collaborator facilities. Within this period basic statistical and computational skills will be developed (if required) by making use of the excellent training facilities at Oxford. The remainder of the DPhil will be used to apply these skill sets to longitudinal trephine samples obtained from clinical trials, such as immune therapies or novel reagents in early phase clinical trials. Biobanked samples will be obtained through close collaboration with clinical collaborator Ramasamy at OTMC.

In situ analysis of MM trephine biopsies and targeted protein marker detection: To this end we have created and validated a targeted proteomic-based affinity reagent panel for deployment in cycling immunofluorescence and/or imaging mass cytometry (IMC) studies. The panel is based on our previous marker selections to characterise the immune environment (myeloid, lymphoid compartments; immune activation, exhaustion) and in addition allows to identify key myeloma factors (such as targets of immune and/or IMID therapies); in addition it provides the ability to determine stromal cell types (such as fibroblasts, endothelial and mesenchymal cells, osteoblasts, adipocytes) in formalin-fixed paraffin embedded (FFPE) trephine biopsies. Data analysis will be carried out by using software packages such as HALO, successfully used in the group. The outcome of this workflow is the characterisation of regions of interest (i.e. regions that attract or repulse immune cell infiltration and activation) in trephine samples; subsequent histological sections of the same sample will then be used to perform laser caption dissection followed by targeted genome sequencing and proteomic analysis of the region of interest.

Targeted genome sequencing analysis using the recently developed myeloma genome panel developed by co-applicant Thakurta[3] will be applied by the DPhil candidate on DNA from matching FFPE sections using laser capture microdissection using an extraction-free library preparation technique developed by collaborator Rao[4]. Depending on the number of tumour cells, we will subdivide the tumour into subregions and individually barcode them. We will thus be able to obtain copy number alteration and single nucleotide variant information from spatially distinct regions of the biopsies, which we will integrate with proteomic data from adjacent sections.

Mass spectrometry based proteomic analysis of the myeloma tumour and its environment will be in collaboration with Professors Kessler and Fischer at the Centre for Medicine Discovery (CMD), who have co-developed and applied in situ mass spectrometry workflows[5]. They will supervise the student in laser capture microscopy and mass spectrometry techniques as well as data processing and analysis.

Integration and data analysis – we anticipate that by combining the information and multiple datasets with the spatial distribution of immune and bone cells relative to the tumour cells plus the clinical data will provide significant novel insights into multiple myeloma pathobiology. Co-supervisor Cribbs has developed workflows for dataset integration and will work with the DPhil candidate to apply machine learning techniques in order to identify patterns that define tumour – microenvironment interactions and therapeutic response.

Translational potential

We expect that this project will significantly increase insights from our previous genomic and transcriptomic analyses of myeloma bone marrow aspirates by applying spatial omics analyses, including genomics and global proteomics. To our knowledge this is a pioneering attempt in the myeloma field and we foresee that a systems-based approach will enable us to answer the following research questions: what are the cellular components (immune, stromal cells) that infiltrate or surround the tumour? What is the activation/exhaustion status of the immune cells that are found at the tumour site? Does the genomic background play a role in the immune response or the spatial parameters? Can therapeutic responses and mode of action be gained from spatial information?

The translational value lies in (i) a better understanding of the heterogeneity of MM and how this is related to spatial interactions and tumour phenotypes (such as proliferation, immune exhaustion, evasion), and (ii) the possibility to identify therapeutic intervention points that can be utilized to inform on adequate therapies to achieve desired responses, especially in relapsed/refractory settings. Furthermore, we expect that (iii) LCM of the MM tumour and its immune and stromal environment will provide essential and novel information about how tumour cells communicate with their non-tumour environment, which possibly provides novel avenues in immuno-oncology research.

Training opportunities

The DPhil candidate will receive training in wet lab activities covering multiple of aspects of modern cell and molecular biology (next generation sequencing, state of the art proteomics, metabolomics, imaging (cycling immunofluorescence, imaging mass cytometry) as well as training in computational and statistical methods. The Oxford Translational Myeloma Centre is an ideal place for interested candidates either with a medical training (with a required interest in haemato-oncology) wishing to deep-dive into state-of-the-art multiomics and computational techniques or molecular biologists wishing to expand their skill sets and apply these to myeloma research. Training of next-generation fellows is a key aspiration of OTMC. Ample opportunity is provided to successful candidates to interact with pharmaceutical industry, due to multiple existing research projects of the investigators and collaborators of this proposal.

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20. Do mutations in cancer arise through histone post-translational modifications? [1,2,3,4] – Assoc Prof. Peter Sarkies

Primary Supervisor: Assoc Prof. Peter Sarkies
Additional Supervisors: Assoc Prof. Benjamin Schuster-Böckler
Eligibility: All tracks are eligible to apply for this project.

Abstract
Mutations in the sequence of DNA are the fundamental source of cancer, leading to activation of oncogenes and inactivation of tumour suppressor genes[1]. Almost all cancers demonstrate genomic instability with mutation rates greatly elevated above healthy cells; indeed, genomic instability is widely accepted as an enabling characteristic supporting cancer growth by increasing the phenotypic diversity of cells within a cancer and providing ways in which cancers can become more aggressive by evading the immune system, or initiating metastasis [2]. Over the last 10 years, high throughput sequencing of primary human cancers has provided a spectacularly detailed characterisation of the mutations that define cancers and identified a number of mutational "signatures" that together can be used to classify mutational processes [3]. Some mutational signatures can be assigned to specific cellular processes [4] or exogenous agents such as tobacco smoke[5]. However, the vast majority are still unexplained [6]. It is therefore key to our understanding of cancer to develop improved insight into the sources of these mutational signatures. Mutations often arise as a consequence of damage to DNA. The Sarkies lab discovered that DNA methylation, a key form of epigenetic regulation in human cells, also has the potential to damage DNA through off-target alkylation of cytosine[7,8]. Left unrepaired, this can lead directly to mutations[9]. Epigenetic regulation beyond DNA methylation, in particular histone post-translational modifications, takes place close to the DNA and, like DNA methylation, uses metabolically active co-factors. We therefore hypothesise that histone post-translational modifications could be a hitherto unknown source of DNA damage and thus account for some of the unclassified mutational signatures in cancer.

The aim of this joint project is to test this hypothesis by combining expertise from the two groups. The first stage will take place in the Sarkies laboratory, which specialises in using evolutionary approaches to understand epigenetic regulation. We will use co-evolution analysis across eukaryotic species as well as co-expression analysis from human cancers and healthy tissues to identify potential associations between histone post-translational modification rates and specific DNA repair pathway activity. This will give clues as to how histone post-translational modification could damage DNA. In the second part of the project, using the expertise of the Schuster-Böckler laboratory (e.g. [10]), the student will test whether the links between histone post-translational modifications and DNA repair pathways can explain mutational signatures using state-of-the-art machine learning methods to analyse high-throughput sequencing data. Finally, we will test our hypotheses in the laboratory by using experimental introduction of histone modifications into specific sites in human Acute Myeloid Leukaemia (AML) cells to test if this incurs increased mutations in the same region. Together this will enable us to identify novel sources of endogenous DNA damage due to epigenetic processes. Crucially, this will not only enable us to better understand how cancer arises, but also to predict which cancers will be particularly vulnerable to specific chemotherapeutic agents, as cells with high levels of histone post-translational modification induced DNA damage will be more vulnerable to agents that introduce the same type of damage.

Research objectives

Academic value
Mutational signatures underpin how cancers develop. Understanding where different mutational signatures come from, however, is much harder than classifying them. Our hypothesis that histone post-translational modifications might be responsible for some of the many "orphan" signatures therefore has the potential to provide important new insight into cancer development. Additionally, our research may provide new insights into why epigenetic pathways are so frequently perturbed in cancer. Together, these will provide new avenues to explore the fundamental basis of cancer development and progression.

Collaborative value
The Sarkies lab is based in the Biochemistry Department and is focussed on using evolutionary methods to understand epigenetic regulation. The Schuster-Böckler lab is based in the Ludwig Department of Cancer and focusses on computational analysis of molecular processes in cancer. This will therefore bridge two different departments and link two labs with disparate interests and expertise. Sarkies and Schuster-Böckler are two early career group leaders, and have

never collaborated together. Supervising a joint student is a direct way to promote collaboration and establish a new and lasting collaboration between the two research groups.

Translational potential

The key translational outcome is in the arena of better targeting of chemotherapies. Crucially, many chemotherapy treatments work by introducing DNA damage. If we can better understand sources of DNA damage due to epigenetic pathways such as histone post-translational modifications it will enable us to predict, from the activity of these pathways, which cancers will be more vulnerable to particular DNA damaging agents. If we discover that a particular histone post-translational modification promotes DNA alkylation damage, tumours with high levels of this modification could be targeted for treatment with alkylating agents. Since measuring histone post-translational modification levels is straightforward, such a targeted approach would be an attractive and feasible outcome of the research described in this proposal.

Training opportunities

The project will provide training in comparative genomics across species and gene expression network construction and analysis in cancer. It will provide training in machine-learning methods and modelling approaches for mutational signature identification. It will also provide training in basic molecular biology techniques, including cell culture, CRISPR and high-throughput sequencing.

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21. Characterizing the tumour microenvironment of oesophageal cancer to uncover key factors in response to immunotherapy [1,2,3] – Carol Leung & Prof. Benoit Van den Eynde

Primary Supervisor: Carol Leung & Prof. Benoit Van den Eynde
Additional Supervisors: Assoc Prof. Eileen Parkes
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Abstract
With the advent of immune checkpoint blockade as standard of care in many cancers, such as oesophageal adenocarcinoma (OAC), there is now a pressing need to identify new combination strategies for the very high proportion of immune checkpoint blockade-resistant or non-responding diseases. OAC is a solid cancer of unmet needs, bound to affect increasing numbers of people and overall poorly characterised in terms of tumour microenvironment. Unlike other solid tumours, OAC offers relatively easy access to the primary tissue, making it an ideal cancer type for microenvironment studies. OAC is characterised by chromosomal instability, with highly chromosomally unstable (CIN-high) OAC carrying a particularly poor prognosis, associated with treatment resistance. CIN-high OAC has high expression of genes involved in organisation and remodelling of the extracellular matrix, which determines the structure of the tumour and induces a distinct fibroblast phenotype within the microenvironment. How this in turn affects the immune compartment of CIN-high OAC cancers remains to be characterised. In addition, the link between CIN-status, tumour microenvironment and response to immunotherapy has still to be explored.

Research objectives and proposed outcomes

Here we will investigate the tumour microenvironment of CIN-high and CIN-low OAC using fresh tissue biopsies and blood collected from OAC patients before and after treatment with immune checkpoint blockade combinations. The aims are:

- *Profile the immune components in the tumour microenvironment of CIN-high and CIN-low OAC*. Using multiparameter flow cytometry, fresh and untreated OAC tissue and blood samples will be processed and analysed, to phenotype the tumour microenvironment, both in terms of immune cells and fibroblasts. We have previously optimised flow cytometry panels to be employed in this aim. To complement this, a functional assessment of the myeloid compartment within the microenvironment will be performed, in order to determine the pro-tumour or anti-tumour role of such a plastic subset of cells (i.e. their ability to suppress T cell proliferation). This will be the first time the tumour microenvironment in OAC has been profiled and phenotyped in such depth. Collaborative partners in this aim include OUH surgeons and gastroenterologists, as well as the tissue banking team.
  Outcome: Characterisation of the tumour microenvironment in OAC and identification of distinctive immune phenotypes in CIN-high vs. CIN-low disease.
- *OAC tumour microenvironment 3D modelling*. Organoids and matched fibroblasts were previously established from patient samples (Parkes lab). Building on this, we aim to obtain 3D co-cultures to include the myeloid compartment previously characterised (in aim 1) and represent a more accurate OAC tumour microenvironment. This will help dissect the complex crosstalk between the tumour cells, the fibroblasts and the immune cells. Healthy monocytes and granulocytes will be added to organoid + fibroblast cultures and the following questions will be asked: is the CIN-type and fibroblast phenotype inducing polarisation of the myeloid cells to become tumour-promoting, i.e. are these cells immunosuppressive after co-culture? What mechanisms underlie this polarisation event (e.g. cytokines secretion, contact dependent)?
  Outcome: Novel organoid-based co-culture assays to characterise the interaction between myeloid cells, fibroblasts and OAC tumour cells.
- *Interrogate the changes in tumour microenvironment upon treatment with immune checkpoint blockade.* Pre-treatment biopsies as well as matched post-treatment samples will be compared in terms of tumour microenvironment profile and transcriptomics, in order to assess the changes occurred within the tumour microenvironment as a result of immune checkpoint-blockade therapy, with particular attention to checkpoint blockade-resistant OAC. The overarching goal is the identification and validation of tumour microenvironment pathways to target alongside standard of care as a foundation for combination early phase studies.

Translational potential

This proposal will identify and characterise new therapeutic opportunities in the immuno-oncology space by dissecting the interactions between tumour and its microenvironment immune checkpoint blockade-resistant OAC. Targeting the tumour microenvironment addresses an urgent barrier to successful immune recognition of cancer cells, and as such supports the future development of combination immuno-oncology strategies. Moreover, the characterisation of the microenvironment of CIN-high OAC will inform on approaches potentially relevant to other CIN-high solid cancers.

Training opportunities

The successful applicant will be co-supervised by Dr. Carol Leung, Dr. Eileen Parkes and Prof. Benoit Van den Eynde. The student/clinical researcher will be given training in immunology and molecular biology techniques including flow cytometry, 3D culturing of organoid models, primary tissue processing, cell sorting, digital pathology analysis as well as bioinformatic training for RNAseq analysis. The Van den Eynde and Parkes labs are established teams including postdoctoral fellows, DPhil students as well as technical support. Hands on training and support will be provided for all the techniques outlined. Students will also have the opportunity to attend bioinformatics training to learn or advance their bioinformatic skills. In addition to the scientific aspect of the research project, the student/clinical researcher will benefit enormously from the career development programme at the Ludwig Institute.

Cancer Centre: This project has a distinct focus on **immuno-oncology,** a key theme in cancer research. This includes novel collaborations involving two research groups, in the Ludwig Institute for Cancer Research, and the Department of Oncology, promoting a cancer centre-wide approach. This project focuses on oesophageal adenocarcinoma, a digestive tract cancer of unmet need, with the potential to translate benefits onto other solid cancers

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22. Tertiary Lymphoid Structures in Lung Cancer [1,2,3] - Dr. Isabela Pedroza-Pacheco

Primary Supervisor: Dr. Isabela Pedroza-Pacheco
Additional Supervisors: Assoc Prof. Eileen Parkes
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Abstract
Organised collections of immune cells, called Tertiary Lymphoid Structures (TLS) are identified in multiple cancer types. In lung cancer, B cell-rich TLS near tumours are strong predictors of immune checkpoint blockade response in lung adenocarcinomas. TLS mimic the organisational and structural features of secondary lymphoid organs. TLS are thought to act as a local source of immune cell recruitment for rapid immune response and therefore, play an important role in promoting and maintaining anti-cancer immune responses. Studies of TLS in established cancers have suggested that TLS can be induced by chemotherapy or vaccination – although why TLS are induced in some patients and not others, or the consequent role they may play in orchestrating immune responses, remains unclear. Here we use a novel in vivo model in which we can reliably generate both lung tumours and the tertiary lymphoid structures to interrogate the relationship of TLS with early tumorigenesis.

Research objectives

- **Investigation of the timing of TLS formation alongside lung cancer development**

Here, a time course experiment will be performed. We will use our established methods to establish mice treated to develop cancer and/or organised tertiary lymphoid structures. Left lungs will be profiled using *multiplex immunofluorescence*, using our established panel. Spatial relationship of immune cells and TLS to developing tumours as well as the composition of TLS over time will be analysed using digital pathology with features identified in association with tumour growth/regression. Remaining right lobes will be digested and profiled using state-of-the-art *high-dimensional spectral flow cytometry analysis* for high-throughput protein-based screening of myeloid, T-cells, B-cell/antigen presenting cell subsets, and natural killer cells. Mediastinal lung-draining lymph nodes from each mouse will be profiled similarly. This 38-multiplex spectral flow cytometry panel has been developed and validated uniquely in-house by Dr Isabela Pedroza-Pacheco and is a low-cost high-throughput immunophenotyping approach.

Outcomes: This approach will address the role of TLS in early tumorigenesis and identify factors driving TLS neogenesis that could be exploited therapeutically. Immunophenotypes and novel TLS signatures associated with effective and sustained immune responses will be defined.

- **Characterisation of tumour-controlling TLS to understand factors driving effective anti-cancer immunity**

Mature TLS with a GC are reportedly most effective at promoting tumour immune recognition in established tumours. Our preliminary data demonstrates that there is marked variation between the number, size, and appearance of TLS associated with tumour control. TLS characteristics that could promote recognition and elimination of precancerous/cancerous cells are currently unknown. Therefore, in this work package, spatial transcriptomics will be used to identify distinguishing features of TLS that develop in the absence/reduction of tumour growth and those which occur alongside tumour growth. We will use fresh frozen samples from mice at endpoint (~28 weeks post urethane injection) and profiled using the NanoString GeoMx platform. TLS for profiling will be selected using features of size, organisation, presence of germinal centres and proximity to tumours for elution and whole transcriptome sequencing. Up to 40 regions of interest across each of 5 conditions will be profiled.

Outcomes: Defining TLS features that are associated with tumour control, regression or prevention will enable development of future therapeutic approaches promoting effective and sustained immune responses.

- **Define the spatio-temporal distinctions in antigenic landscape driving TLS formation and maintenance**

Whilst it is thought that effective antigen presentation and anti-tumour responses are facilitated by TLS, it is not known if antigen presentation within TLS, or in the presence of TLS, differs from antigen presentation in tumour tissue. Moreover, detailed study of antigens presented during tumorigenesis and TLS formation using longitudinal samples has not previously been possible. Defining this changing landscape would enable identification of antigens that may be potently and distinctly capable of driving TLS formation and effective immune responses in early stages of cancer. Mass spectrometry (MS)-based immunopeptidomics is currently the only method that enables the direct identification of MHC-bound peptide antigens. Here, we will use a novel in-house established timsTOF workflow [for] increased sensitivity **interrogating the MHC-I and MHC-II antigen landscape and temporal relationship with cancer** in TLS-rich and TLS-low/absent tumour lung tissue.

Outcomes: These data will identify the antigens presented over the course of tumour development as well as in TLS-poor and TLS-rich lung cancers. Therefore, the distinct immunopeptidome of TLS can be discerned and relationship to effective anti-cancer immunity pinpointed.

Translational potential

To date, TLS have been studied in established cancers, not in the context of tumorigenesis. Therefore, the factors driving TLS formation, maturation and maintenance and their role in enhancement of immune response in early tumour development are not known. This study will use a novel and tractable *in vivo* modelling approach that facilitates the development of TLS alongside lung cancer. In-depth characterisation will mechanistically dissect predictive cellular and molecular factors driving effective anti-tumour immune responses. This will identify TLS-specific factors which enable tumour control or escape, with future therapeutic relevance.

Training opportunities

In vivo modelling, Flow cytometry analysis, multiplex immunofluorescence, digital spatial profiling, bioinformatics techniques include R for analysis of transcriptomic data, immunepeptidomic analysis.

References

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23. Interrogating the fibroblast phenotype of DNA repair deficient cancer [1,2,3] – Assoc Prof. Eileen Parkes

Primary Supervisor: Assoc Prof. Eileen Parkes
Additional Supervisors: Prof. Kim Midwood
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Abstract
Understanding the tumour microenvironment of BRCA1-mutant breast cancer is key to developing new therapeutic strategies to improve durable responses to treatment. Cancer-associated fibroblasts (CAFs) are not merely by- standers in the tumour microenvironment, but crucial in treatment response and metastasis. Using models of this disease subtype developed in our lab we have identified a unique CAF phenotype potentially associated with inhibitor resistance. In this proposal we will investigate how CAF phenotype may impact response to PARP inhibition, and further investigate the CAF phenotype in BRCA1-mutant breast cancer.

CAFs are the dominant component of the stromal tumour microenvironment and contribute to cancer progression as well as therapeutic response. CAFs contribute to an immune-excluded and immunosuppressed tumour microenvironment as well as driving pro-tumorigenic macrophage polarisation. Recently it has been reported that tumorigenesis in *BRCA1*-mutant cancer is partly driven by a distinct fibroblast population. Despite this crucial role, the phenotype of CAFs in *BRCA*-deficient breast cancer and their contribution to therapeutic response/resistance, particularly to PARP inhibition, is poorly understood.

Research objectives

- **Investigation of response to PARP inhibitor treatment and impact of fibroblast phenotype in BRCA1-mutant and wildtype *in vivo* models**

Our preliminary data demonstrates the distinct CAF phenotype in *Brca1*-deficient treatment-naïve tumours. This aim will further characterise the impact of PARP inhibition on fibroblast phenotype using FACS immune profiling. Using the 4T1 *Brca1* isogenic models, we will perform *in vivo* treatment with the PARP inhibitor niraparib. Tumours will be resected and half the tumour dissociated and digested for subsequent FACS analysis, using lymphoid (CD3, CD4, CD8, CD19, NKp46 and CD279), myeloid (CD80, MHS II, Ly-6G, Ly-6C, CD11b, CD11c, CD206, F4/80) and fibroblast (panCK, CD31, PDGFRα, FSP1, SMA, Podoplanin, MHCII) antibody panels.

Outcomes: These experimental approaches will further address the distinct CAF phenotype in the 4T1 sh*Brca1* model as well as identifying the impact of PARP inhibition on CAFs in the tumour microenvironment, an important unanswered question.

- **Characterisation of fibroblast phenotype in human breast cancer using spatial transcriptomics**

While our preliminary data strongly supports a distinct CAF phenotype in the murine models we have studied, we do not yet know the translational relevance of this to human breast cancer. In collaboration with the Breast Cancer Now biobank we have received preliminary approval (see letter of support) to obtain formalin-fixed paraffin- embedded samples of triple negative breast cancer from patients with *BRCA1* mutation (n = 4) and from *BRCA1* wildtype tumours (n = 4). Tumours will be matched for size (T) and nodal status (N) stage as well as histological subtype (ductal cancer) to minimise confounding variables. Whole face sections (a single 4 µm section from each tumour) will be stained with DAPI, CD45+ (immune cells), SMA (CAFs) and panCK (tumour cells). Regions of interest will be selected using the GeoMx platform. Downstream Illumina NGS capturing whole transcriptome will then be performed on distinct regions of interest, with in depth bioinformatic analysis performed.

Outcomes: Identifying the ability of murine models to model CAFs in *BRCA*-mutant breast cancer will address the translatability of the findings of this study – whether *in vivo* models are sufficient to investigate potential combination strategies or alternative approaches (e.g. PDX, patient-derived organoids and co-culture) are needed to accurately model and optimise clinical impact.

- **Modelling fibroblast phenotype to investigate how fibroblasts impact tumour growth, therapeutic response and metastasis in breast cancer**

From our preliminary data analysis (RNAseq of fibroblasts from 4T1 *Brca1* isogenic tumours) and further data obtained in aim (2), consensus hits between mouse and human tumours will be identified and ranked. Top hits identified will be genetically manipulated on GFP-labelled 3T3 Balb/c fibroblasts (in house) using lentivirus CRISPR Cas9 (LentiCRISPR v2, in house). We will use conditioned media from 4T1 and 4T1 sh*Brca1* cells *in vitro* and perform 10 – 14 days co- culture with fibroblasts to determine the direct impact of chemokines and conditioned media on fibroblast plasticity. Conditioned media from genetically manipulated fibroblasts will additionally be co-cultured with bone marrow derived monocytes, derived from bone marrow flushes of humanely killed Balb/c mice, to determine impact of fibroblast phenotype on myeloid cell differentiation and phenotype, analysed using FACS.

Outcomes: Together, these data will identify the role of distinct fibroblast population in tumour growth and spread, and potential therapeutic opportunities to directly target fibroblasts to improve patient outcomes in *BRCA*-mutant breast cancer.

Translational potential

The direct comparison of hits from an *in vivo* model and human breast cancer samples will be impactful in determining how efficiently mouse models can be used to develop therapeutic strategies around CAFs and what limitations there might be of this approach. Overall, it is intended that this work will lead to discovery of potential novel CAF-related targets that could be further validated for clinical use, and accelerate translation to the clinical setting by identifying potential CAF phenotypes that predict response/resistance to PARP inhibition and thus patient selection for alternative or combination strategies.

Training opportunities

In vivo modelling, Flow cytometry analysis, multiplex immunofluorescence, digital spatial profiling, CRISPR/Cas9, cell culture, bioinformatics techniques include R for analysis of transcriptomic data.

References

van Vugt MATM, Parkes EE. When breaks get hot: inflammatory signaling in BRCA1/2-mutant cancers. *Trends Cancer*. 2022;8(3):174-189. doi:10.1016/j.trecan.2021.12.003

24. Exploring the biological drivers of 1q21+ high risk Myeloma by using multi-OMICS analysis of patient derived tumour, immune cells and bone marrow aspirates[1,2,3,4] – Prof. Anjan Thakurta

Primary Supervisor: Prof. Anjan Thakurta
Additional Supervisors: Assoc Prof. Karthik Ramasamy
Eligibility: All tracks are eligible to apply for this project.

Abstract

Multiple Myeloma is an incurable malignancy of the clonally expanded plasma cells and is genomically heterogeneous. Up to 40% of newly diagnosed (NDMM) and 70% of relapsed and refractory Multiple Myeloma (RRMM) patients carry gain or amplification in chromosome 1q21 locus. Copy number gain (3 copies) or amplification (4+ copies) together designated as 1q21+ are poor prognostic biomarkers in NDMM and feature prominently in driving disease progression in the relapse settings. Various groups have previously analysed large datasets to define the prognosis of 1q21 MM. Notable among these, the analysis from the Myeloma Genome Project (MGP), co-led by the applicant, identified amplification of 1q21 in the background of ISS3 (international staging system) as a 'double hit' MM with very poor clinical outcome.1 Another analysis of 1905 patients from the Myeloma UK group interrogated a combined dataset from Myeloma X and XI trials and confirmed the poor prognosis of 1q21. This analysis also demonstrated co-occurrence of genomic features to add adverse prognosis of this group of MM patients.2 Recently, by paired tumour sample analysis, we have found genetic lesions of 1p32LOH (loss of heterozygosity) with 1q21 gain co-evolve in driving clinical resistance in Myeloma (Ansari-Pour, Thakurta, unpublished results). There are many postulated biological drivers and therapeutic targets of MM within the 1q21 locus that are being analysed and pursued. Previously, by unsupervised analysis of the genome and transcriptome of 514 NDMM patients, we identified a biological subset of patients (designated as MDMS8) with poor prognosis3. While diverse high-risk genomic features (including ~ a third with 1q21 gain) were identified, we found a common transcriptional program among the patients of this subset. The tumour cells in this group showed significant loss (~ 10%) of genome, damaged DNA repair pathways, dysregulated cell cycle as key genomic and transcriptomic features of high-risk disease. Master regulators were identified as key drivers of the transcription program. However, due to lack of availability of samples, a detailed proteomic analysis was not performed to interrogate the functional and metabolic drivers of high-risk MM.

Analysis of Myeloma proteome (and metabolome) is thus far limited but some published proteomics literature suggests its feasibility and the potential utility. For example, by analysing 5 paired patient samples Ng et al identified CDK6 as a driver of resistance to immunomodulatory drugs4. Proteomic analysis of bortezomib sensitive and resistant patient samples similarly led to the discovery of a potential biomarker of resistance5. Another group explored comparative proteomic analysis of bone marrow aspirates and serum to identify new biomarkers for MM5. Metabolic analysis of MM has not been published yet, offering a new opportunity to initiate initial exploration in the study of a high-risk disease subset such as 1q21+ MM. Our hypothesis is that a systematic proteomic analysis of 1q21+ MM cell lines and patient samples may add to the overall understanding of key proteins and biological processes or their regulation that functionally drive the disease. In a separate project, we are exploring genomic drivers of 1q21+ MM for identifying therapeutic targets. Here, the proposed project takes a complementary approach and explores a proteomics-based analysis of patient tumour cells, immune cells and the bone marrow aspirates to establish proteomic and metabolomic profiles of 1q21 MM and validate key functional drivers of high-risk disease.

Research objectives

The project will be divided in terms of experimental work done at Botnar Lab (in collaboration with Prof Oppermann) and Centre for Medicines Discovery, Oxford (with Prof Benedikt Kessler's group), at Metabolon (in collaboration with Dr Sarangarajan) and computational analysis will be done within OTMC (with support from a computational science team). We will access cell lines (Sarah Gooding, Oxford Oppermann, Oxford, commercial sources), and patient bone marrow aspirates (Oxford Biobank supervised by Prof Ramasamy) for 1q21+ and 1q21- (standard risk patients) and separate CD138+ and CD138- fractions and use them for proteomic/metabolomic analysis. In agreement with the collaborators, some data or materials from a companion project could be shared for this project work (Dr Erin Flynt). This project will be run in three phases: *Phase I:* In the initial phase, pilot experiments will be set up to study Myeloma cell lines representing normal, 1q21 gain and 1q21 amplified myeloma cells. Alongside, proteomics analysis of immune cell types (CD4, CD8 cells and NK cells) and serum will be performed from peripheral blood from healthy donors.

Total proteome, phospho proteome and metabolic profiles (in collaboration with Metabolon) will be generated from the cell lines to develop the methods for experimental pre-analytics and bioinformatic analytics. A datahub in OTMC will be set up for storage and analysis of data. Various experimental conditions will be tested to mimic real life conditions of patient derived bone marrow aspirates. CytoF based analysis will be conducted for comparing some markers from the immune subsets and Myeloma cell lines using panels already in use in the Botnar lab. Analysis of the cell line data will be used as a benchmark when analysis of patient samples is tested. Once the pilot experiments are completed, based on the analysis, a subset of cell lines will be chosen to perform drug treatments to understand the impact on the proteome and metabolome. Standard of care therapeutic agents such as steroids, immunomodulatory drugs, proteasome inhibitors and anti-CD38 antibodies will be used alone and in clinically used combinations. The final outputs of this work will be the identification of protein(s) affected in 1q21+ Myeloma and/or via drug treatment with potential biological or clinical impact. *Phase 2:* This phase is based on the analysis of 30-40 patient samples in each control or 1q21 + group and further analysis of preclinical data and correlative analysis of potential biomarkers. The key types of questions we will pursue are: What are the specific proteomic/phospho-proteomic and metabolic profiles of 1q21+ Myeloma in CD138+ cells, immune subsets or in bone marrow aspirates compared to normal counterparts? What are the biological drivers of this segment of vs those without (in previously identified genomic high risk MDMS8 segment with 1q+ vs 1q- counterpart). Biobanked and genomically characterized patent samples will be used for this analysis. *Phase 3:* Validation of key proteins or metabolites identified from preclinical and clinical samples will be the focus in this phase. A subset of the most promising candidates from CD138+, bone marrow aspirate or immune cells will be selected. For preclinical validation, cell lines will be modified to create knock downs or CRISPR mediated knock outs, for expression of specific mutants, based on targeted genes and the phenotypic and biochemical effects will be measured. Target feasibility/ targetability analysis is outside the scope of this project.

Translational potential

This project is forward and reverse translational in nature whereby the student will explore preclinical data analysis coupled with retrospective analysis of patient data to derive insights for the development of biomarkers or therapeutic targets relevant for the 1q21+ high-risk Myeloma. The project is planned to run side by side the genomics oriented collaborative project to cross fertilize both projects. The role of 1q21 in relapse in Myeloma is well documented and there is also a clear need to find actionable insights for detection and characterisation of 1q associated biomarkers. There is clear potential to develop a personalised treatment approach to test translationally driven clinical concepts within the OTMC Myeloma Master protocol. This project will bring in an approach so far not addressed by genomics and other approaches.

Training opportunities

The student will learn basic molecular techniques, cell biology methods, some OMICS techniques, data generation and computational analysis of large datasets from multiple sources. The student will also learn the relevant methods of diagnosis and treatment of myeloma and other related OTMC's projects (such as single cell analysis, long read sequencing etc.)

References:

1. Walker BA, et al. A high-risk, Double-Hit, group of newly diagnosed myeloma identified by genomic analysis. Leukemia. 2019. 33(1):159-170.
2. Shah V, et al. Prediction of outcome in newly diagnosed myeloma: a meta-analysis of the molecular profiles of 1905 trial patients. Leukemia. 2018;32(1):102-110
3. Ortiz Estevez, M., et al. Integrative multi omics identifies high-risk multiple myeloma subgroup associated with significant DNA loss and dysregulated DNA repair and cell cycle pathways. BMC Medical Genomics; 2021; 14:295-302
4. Chen, Y., et al. 2021; J. Proteome Res. 2021, 20, 5, 2673–268.
5. Ng, Y., et al. Nature Comm. 2022; 13:1009 https://doi.org/10.1038/s41467-022-28515-1
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25. Development of Spatially Fractionated Radiotherapy Techniques [1] – Assoc Prof. Geoff Higgins

Primary Supervisor: Prof. Anjan Thakurta
Additional Supervisors: Assoc Prof. Karthik Ramasamy
Eligibility: Track 1 students are eligible to apply for this project.

Abstract
Conventional fractionated radiotherapy involves the homogenous delivery of radiation across the entire tumour in 2 Gy fractions. Recently, there has been significant interest in delivering non-uniform "spatially fractionated" radiotherapy (SFRT) particularly for bulky, locally advanced tumours that usually do not respond well to conventional radiotherapy, without increasing the side-effects of treatment. SFRT typically involves delivering extremely high doses of radiotherapy (up to 20 Gy) to multiple, small spherical subregions within the tumour, whilst ensuring that the dose to the periphery of the tumour, and to the surrounding organs at risk are within standard limits. It has been postulated that the high tumour control rates that have been reported with SFRT might be due to anti-tumour immune responses, triggered bystander effects, or changes in the tumour vasculature caused by the peak radiation doses.

The advent of Magnetic Resonance Linear Accelerators (MRL) has increased the ability to accurately deliver radiotherapy to moving tumours and facilitate real time treatment adaptation. This proposal seeks to implement SFRT using a MRL machine. In addition, we will seek to further develop the capabilities of MRL technology by assessing whether low field strength MR imaging is able to effectively identify regions of tumour hypoxia, using a relatively new technique called oxygen-enhanced MR imaging.

Research objectives

The Oxford Institute for Radiation Oncology has a well-established partnership with GenesisCare that provides access to a clinical Viewray MRL which combines a 0.35 Tesla MR scanner with a 6MV photon linear accelerator and offers a potentially ideal opportunity to accurately implement SFRT. This project will seek to assess the capability of delivering SFRT to bulky tumours using the Viewray MRL (Fig 1). In addition, we will investigate the functional imaging capability of the MRL in detecting hypoxic regions within the tumour using oxygen enhanced MR imaging (OE-MRI). This technique has been developed as a non-invasive approach to measure the dynamics of tumour oxygenation. Previous attempts at boosting the dose of radiotherapy to hypoxic regions have not been able to obtain real time assessment of tumour hypoxia, and have only been able to deliver very modest increases in radiotherapy dose. The potential to utilise modern MRL delivery techniques to cause a far greater increase in radiation doses might represent a step change in our ability to overcome hypoxia mediated radio resistance.

The first part of the project will involve radiotherapy planning studies to develop the optimal methodology to deliver MRL based SFRT and will include analysis of the position, size and dose of high dose "spheres".

Subsequently, we will open a clinical study combining SFRT and OE-MRI in patients with locally advanced disease that cannot be treated with radical radiotherapy. This would be expected to include patients with large volume non-small cell lung cancer who are currently treated with high-dose palliative radiotherapy (39 Gy in 13 fractions). This study will also incorporate exploratory imaging biomarkers of early response to treatment, in addition to circulating markers of inflammation, immune activation and tumour response.

This project will centre on an academic collaboration between experts in radiation oncology (Geoff Higgins) and medical physics (Kristoffer Petersson) and will involve detailed radiotherapy planning studies and clinical implementation.

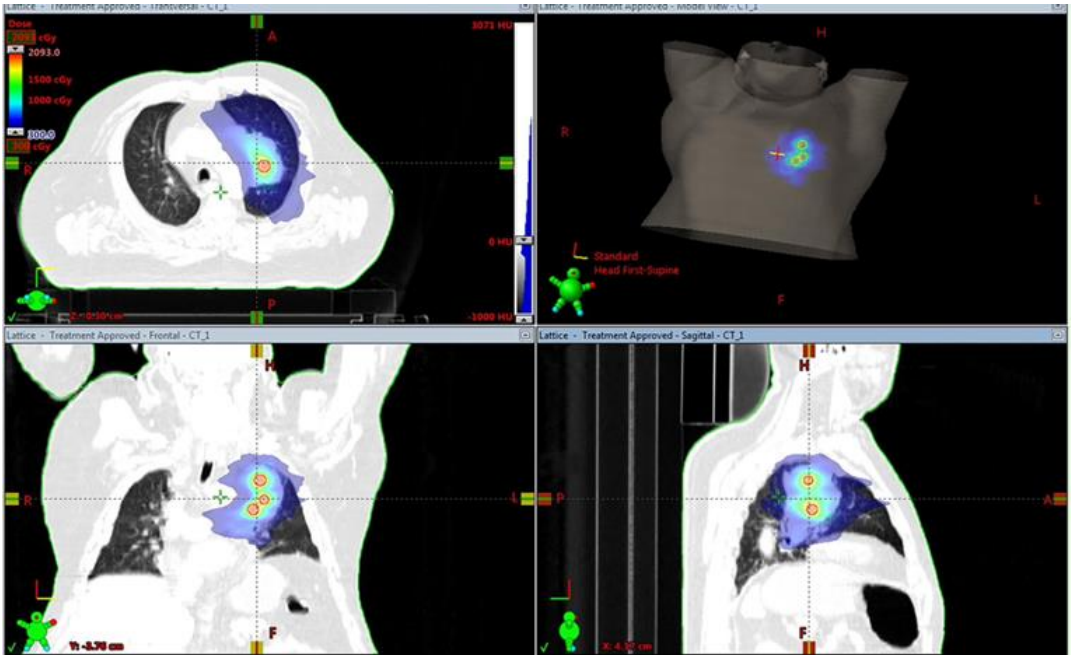

Fig. 1. Illustration of a SFRT treatment plan. Clockwise from top left; axial, model, coronal and sagittal views of colour wash dose distribution of 3 Gy around the tumour periphery and 18 Gy in the three 1.5 cm diameter dose peak spheres within the tumour. Adapted from (3).

Translational potential of the project

The development of MRL machines has advanced the capability to deliver radiotherapy in ways that have not previously been possible. SFRT has the potential to improve radiotherapy outcomes for patients with large tumours that are not amenable to conventional radical radiotherapy and is particularly suitable for MRL based delivery. Combining SFRT with an OE-MRI approach to target hypoxic regions might result in improvements for cancer patients treated with radiotherapy.

Training opportunities

This project is suitable for a clinical oncology trainee interested in pursuing a career as a clinical academic. In addition to the successful candidate gaining expertise in MR guided radiation therapy delivery, they will be also be taught advanced radiotherapy planning methodology, functional MR image analysis and clinical trial experience.

References

1) Yan et al. Spatially fractionated radiation therapy: History, present and the future. CTRO 20: 30-38 (2020).
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5) O'Connor et al. Oxygen-Enhanced MRI Accurately Identifies, Quantifies, and Maps Tumor Hypoxia in Preclinical Cancer Models. Cancer Res. 76(4): 787-95.

26. Investigating the role of hypoxia for the FLASH effect by combining FLASH Radiation with hypoxia-modulated anticancer drugs[1,3,3,4] – Kristoffer Petersson

Primary Supervisor: Kristoffer Petersson
Additional Supervisors: Assoc. Prof Geoff Higgins
Eligibility: All tracks are eligible to apply for this project.

Abstract

FLASH radiotherapy is a novel radiotherapy delivery methodology using ultra-high dose rates (>30-40 Gy/s), ~1000 times higher than what is used clinically[1]. Recent global preclinical research has indicated that FLASH can reduce radiation-induced damage in healthy tissues with similar anti-tumour effects as conventional radiotherapy, the so-called "FLASH effect". Though FLASH clinical trials have started, we do not yet understand the radiobiological mechanism(s) responsible for the FLASH effect. One hypothesis is that FLASH radiation consumes oxygen at a much higher rate than can be re-supplied, causing hypoxia and thereby increased radio-resistance in cells/tissue[2]. Why this is specifically sparing normal tissue has been debated, with one idea being that the tumour cells that are hard to kill with radiation are often already hypoxic, i.e. little change in response to FLASH vs conventional irradiation[1].To test this hypothesis and at the same time test the response of FLASH radiation as a combination treatment with clinical anticancer drugs, we will combine FLASH radiation with Tirapazamine (activated in hypoxia) and Atovaquone (increases tumour oxygenation) in 2D (Tirapazamine, hypoxia and normoxia) and 3D (Spheroids, Tirapazamine and Atovaquone) cell cultures[3]. If the FLASH effect is caused by hypoxia, the FLASH radiation + Tirapazamine should be more toxic than conventional radiation + Tirapazamine. Similarly, the FLASH sparing effect should be reduced when combined with Atovaquone, resulting in similar response as conventional radiation + Atovaquone. To summarise, the response in tumour cells should increase when FLASH is combined with these drugs. However, for a therapeutic benefit it will be important to understand how normal tissue/cells respond to the treatment. For that purpose, we will also expose 3D cell cultures (organoids) to the treatment and evaluate the radiation-induced toxicity. The FLASH-drug combinations that show therapeutic benefit will subsequently be evaluated in mice to further investigate its potential for clinical translation.

Research Objectives

The research project aims to elucidate if the oxygen consumption hypothesis is the main contributor of the FLASH sparing effect seen for normal tissue. By using 2D and 3D cell cultures with controlled levels of oxygen and comparing the response to FLASH vs conventional irradiation with and without drugs that are known to modulate oxygen levels or that activates in hypoxia, we will be able to quantify the importance of oxygen concentration for the FLASH effect. In addition, the FLASH-drug combination that show the highest therapeutic potential will be evaluated in vivo (mice). This will give an indication of the potential of this combined therapy for clinical translation

Translational potential

FLASH radiotherapy has received a lot of research focus recently due to its ability to spare normal tissue, while keeping the tumour response comparable to conventional radiotherapy. Without knowing much about the radiobiological mechanisms responsible for these beneficial characteristics of FLASH, clinical trials have already started. Likely, such studies are not designed in an optimal way as we do not yet know when we see a FLASH effect, how large it is, and why we see it. Hence, we need more preclinical data to support the design of clinical trials and to guide us in the clinical translation of this promising treatment technique. This project will tell us about the role of hypoxia for the FLASH effect and could potentially serve to answer all the questions stated above. In addition, our FLASH-drug combination treatment could help to further widen the therapeutic window beyond what FLASH alone is able to achieve, i.e. further enhancing the biological benefit of the FLASH effect.

Training opportunities

The student will be trained in various in vitro assays, such as Western Blot, DDR analysis, and clonogenic assay. They will also be trained and work with more advanced in vitro models, e.g. spheroids, organoids and 3D bio-printed tissue models. In addition, the student will be trained on the use of in vivo (mouse) models, evaluating normal tissue toxicity and tumour response to treatment. Correct dosimetry is essential in order to get useful and accurate preclinical results from our studies. Dosimetry also becomes more challenging at ultra-high dose rate. Hence, there will be some training opportunities in radiation dosimetry.

References:

1. J.D. Wilson, E.M. Hammond, G.S. Higgins, **K. Petersson**. "Ultra-High Dose Rate (FLASH) Radiotherapy: Silver Bullet or Fool's Gold?" *Front. Oncol.* 9, 1563 (**2020**). 10.3389/fonc.2019.01563

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3. G. Adrian, JL. Ruan, S. Paillas, C.R. Cooper, **K. Petersson**. (2022). "In vitro assays for investigating the FLASH effect". *Expert Reviews in Molecular Medicine* 24, e10, 1–12 (**2022**). https://doi.org/10.1017/erm.2022.5

27. Using Long-read Sequencing to Advance Personalised Decision Making in Multiple Myeloma [1,2,3,4] – Assoc Prof. Adam Cribbs

Primary Supervisor: Assoc Prof. Adam Cribbs
Additional Supervisors: Sarah Gooding
Eligibility: All tracks are eligible to apply for this project.

Abstract
Work within the Cribbs lab focuses on developing novel technology and computational analysis frameworks that empower new modes of treatment for disease. Recently we developed several single-cell and bulk long-read (LR) sequencing approaches to improve their utility and accuracy[1, 2]. This technology allows for the measurement of translocations, variant calling, and alternative splicing in unprecedented detail. We have begun to apply this technology to understand the development of drug resistance in Multiple Myeloma (MM).

We propose that leveraging LR sequencing's multi-modal data can unlock a deeper grasp of functional genomics, refining our diagnosis and prediction of MM with greater precision. Thus, we will employ state-of-the-art machine learning methodologies to identify and integrate the modalities which best predict outcomes. By doing so, we aspire to unearth not only novel diagnostic methods but also discover potential therapeutic targets, thus propelling us further towards our goal of personalised MM patient care.

LR technology
Short-read (SR) sequencing methods face challenges in fully elucidating the intricacies of both DNA and RNA rearrangements. Owing to their capacity to cover thousands of base pairs, long-reads possess the ability to detect mutations in regions where short-reads falter. They excel in identifying complex chromosomal rearrangements, especially those involving numerous chromosomes and heavily repetitive regions[3].

At the RNA level, the challenge lies in accurately documenting alternative splicing events, which can generate a multitude of substrate combinations from the same gene, resulting in thousands of isoforms. Isoform shifts can change drug responses, as we have shown in the response to IMiD drugs in myeloma[4]. Existing SR-sequencing methods are inefficient at capturing this complexity, necessitating the use of LR-sequencing (Fig.1). LR enables end-to-end mRNA sequencing, effectively circumventing these limitations.

Finally, LR sequencing provides the added advantage of simultaneously detecting DNA modifications, such as methylation, in a single experiment, thereby enhancing our understanding of genetic information at multiple levels.

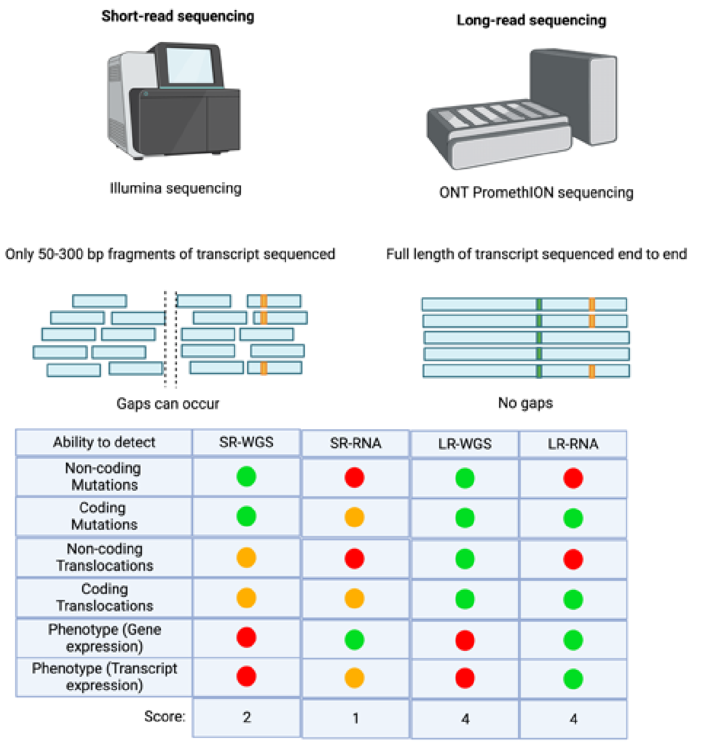

Figure 1: A comparison of the advantages and disadvantages of long-read technologies

Research objectives
Our ultimate aim is to apply our long-read RNA and DNA technology to primary MM patient samples and then generate computational models that help us to understand the molecular mechanisms of drug resistance in patients with relapsed MM.

***Overall aim:* Use LR sequencing data to discover new myeloma characteristics and potential treatment targets.**
With the goal of uncovering unique insights into myeloma biology and pinpointing potential therapeutic targets, we will leverage LR sequencing data to understanding the intricacies of the disease, illuminating novel hallmarks, and thus empowering us to target myeloma more effectively and precisely.

Hypothesis: LR sequencing will give unprecedented detail on the splicesome of MM, largely unstudied, and that can lead to novel drug targeting

We will sequence 600 RNA samples isolated from several ongoing clinical trial samples with external collaborators at UCL, London, at baseline and following relapse. We have already generated pilot data on RNA and DNA isolated from 21 MM patients and identified several novel translocations not identified previously by SR-RNA sequencing (Fig2). We have a well-established wet-lab workflow with which we will generate data on 600 MM patients. It is anticipated that over half of the samples will have been sequenced by the start of the DPhil.

Outputs
- Apply workflow to 400 newly diagnosed and 200 follow-up myeloma patients in a clinical trial.
- Assess the role of alternative splicing in plasma cells in causing disease variation and treatment response.
- Gain new insights into disease mechanisms and treatment responses.
- Potentially influence changes in therapeutic intervention.

***Work package 1:* What do structural changes of MM look like with LR RNA sequencing?**
Analysis of LR-RNAseq: The student will adapt and further develop our established computational analysis workflows (https://github.com/cribbslab/TallyTriN) to facilitate efficient identification of genomic alterations and changes in the transcriptome landscape.

We will use workflows described above to analyse our data focusing on:
- Evaluating changes in Isoform expression before and after relapse.
- Measuring structural changes, mutations and SNPs.
- Exploring relationships in gene expression with copy number variation (CNV)

***Work package 2: Developing a splicosome map of* MM?**
Using the data collected from LR-RNA-Seq, we will develop a comprehensive map of the spliceosome's interactions and RNA modifications across different MM stages. This could provide insights into the heterogeneity and evolution of the disease.

- This analysis will be done in a large phase III MM clinical trial recruiting 1500 patients across the UK. We will focus on early progressors, representing the most unmet medical need.
- Identify potential splicing-driven resistance mechanisms, for example in relation to IMiD exposure.

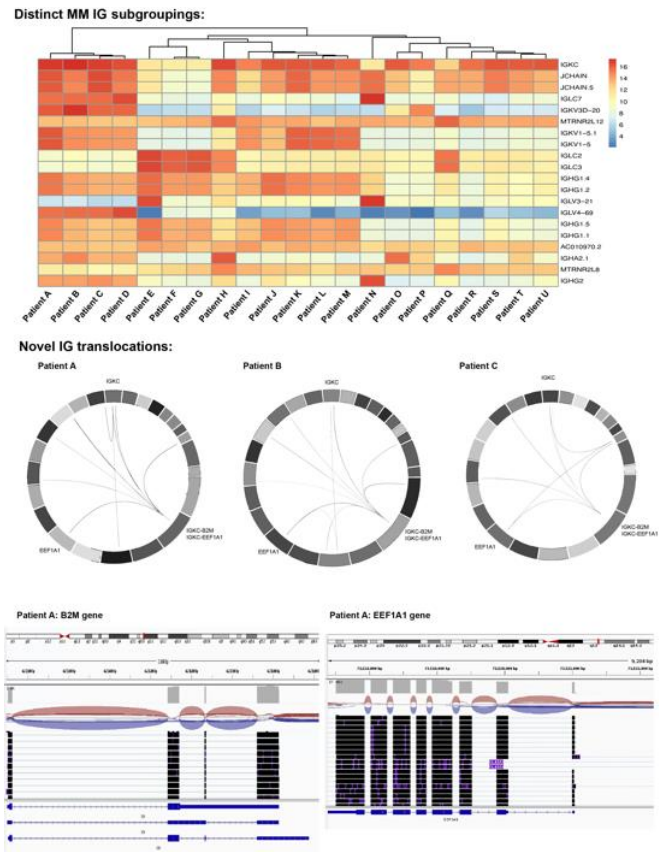

Figure 2: We have identified several novel translocations in RNA isolated from patients with MM.

Translational potential

The stated aim of this project is to study the molecular mechanisms of Multiple Myeloma drug resistance and evaluate drug targets for therapy. By its very definition, this is likely to identify novel therapeutic intervention points within the development of Multiple Myeloma. We have extensive collaborations with several pharmaceutical partners, and we will utilise these interactions to explore the translational potential of targets.

Training opportunities

The student will receive training in the necessary cellular, molecular, and epigenetic biology for this project. This will involve wet-lab workflows for generating LR sequencing data. Extensive training in computational biology will be provided so that the student can analyse their own data. Specifically, this will include software development, data analytics, statistics and computational pipeline development. Outside the lab, the student will be expected to attend regular seminars with high profile external speakers, journal clubs and training in presentation skills, scientific writing, and data management.

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28. The role of oxygen chemosensitivity in tumourgenesis [1,2,3,4] – Prof. Richard White

Primary Supervisor: Prof. Richard White
Additional Supervisors: Professor Sir Peter Ratcliffe and Assoc Prof. Tammie Bishop
Eligibility: All tracks are eligible to apply for this project.

Abstract
Cellular hypoxia impinges on the biology of cancer in multiple ways. One of the least well researched is the role of oxygen chemosensitivity, a classical response to hypoxia that involves depolarization and calcium entry in excitable cells and which is centrally involved in cardiopulmonary and respiratory control[1]. Despite decades of research, understanding of the mode of hypoxic signal generation and transduction is still incompletely understood. Nevertheless, several new lines of evidence point to a role of oxygen chemosensitivity in specific types of cancer:

- activation of a specific component of the transcriptional response to hypoxia mediated by hypoxia inducible factor 2α (HIF-2α) leads to acquisition of oxygen chemosensitivity and a phenotype resembling a rare form of cancer (paragangliomas) in certain tissues
- in Type I cells of the carotid body (a common site of paragangliomas) activation of oxygen chemosensitivity leads to cell proliferation[2].
- small cell lung cancer (SCLC) appears to evolve from neuroepithelial cells of the lung, a relatively uncommon lung cell type that displays oxygen chemosensitivity[3]

Despite these advances, our understanding remains limited, in part because of the difficulty of studying mammalian systems that display oxygen chemosensitivity. This project capitalizes on evidence that the basic oxygen chemosensitivity pathway is conserved in the zebrafish (*Danio rerio*)[4]. This is a model organism optimized for high-resolution imaging coupled with ease of genetic manipulation, and has been increasingly used as a model for cancer. We will exploit the imaging potential of zebrafish to enable rapid molecular genetic analysis of the mechanisms underlying oxygen chemosensitivity and their interface with cell growth and proliferation.

Research objectives
The past several decades have witnessed the elucidation of several key mechanisms by which cells sense oxygen levels. Central to this are HIF-1α and HIF-2α, which are hydroxylated at proline residues by prolyl-hydroxylases, allowing their recognition and ubiquitination by the VHL E3 ubiquitin ligase[5,6]. While this response allows for chronic adaptation to hypoxia, organisms also exhibit a rapid acute response to hypoxia. This is a classical response to hypoxia that involves depolarization and calcium entry in certain excitable cells (e.g. those in the carotid body), which results in a rapid integrated cardiopulmonary response to restore oxygen levels. The sensors underlying this acute chemosensitivity to oxygen remain poorly understood.

Not all cells exhibit acute oxygen chemosensitivity, which may be due to the intrinsic cellular context or due to interactions with cells in the local microenvironment. Recent work from the Bishop and Ratcliffe labs have demonstrated that activation of HIF-2α (encoding EPAS1) leads to acquisition of oxygen chemosensitivity in otherwise insensitive cells such as those in the adrenal medulla, suggesting that this factor is sufficient to endow cells with this phenotype. Interestingly, activation of HIF-2α is also tightly linked to the development of certain types of cancer known as paragangliomas, a rare tumour that often harbours mutations in numerous genes affecting the oxygen sensing pathway, including gain of function mutations in HIF-2α itself, as well as loss-of-function mutations in the main negative regulators of HIF: VHL, PHD2 and PHD1[7]. A high proportion of these mutations occur either as germline alleles or due early somatic mosaic events, indicating there may be a critical developmental window for the development of later disease.

It remains unclear whether the oxygen chemosensitive phenotype is mechanistically linked to the development of these tumours. Understanding this requires a model system that allows us to interrogate both phenotypes, but one that is also amenable to rapid genetic manipulation of genes that are likely to be involved. While mice have been a mainstay of such studies, they are time-consuming and it is difficult to test multiple genes in a scalable manner. For these reasons, this project aims to utilize the unique capabilities of the zebrafish (Danio rerio) to study this problem. The zebrafish is a small, transparent vertebrate that is especially well-suited to imaging and rapid genetic manipulation of specific cell types within ***their native*** microenvironment. The basic oxygen chemosensitivity pathway is conserved in fish, especially in certain neuroendocrine cells (i.e. catecholaminergic cells associated with pharyngeal arch blood vessels or neuroepithelial cells of gills)[4,8]. In addition, the zebrafish is highly amenable to modelling of many different types of cancers using genetic approaches[9].

The first aim of this project will be to study acute oxygen chemosensitivity using GCaMP6f reporter zebrafish. This is a fluorescent transgenic line in which depolarization leads to an increase in intracellular calcium, which can be quantified across the entire body of the animal. We will expose fish to acute hypoxia and use the GCaMP6f reporter as a readout of their response, and then use CRISPR to test candidate genes which we hypothesize may be involved in this response[10-12]. These genes include hif-2α itself, G-protein coupled receptors (rgs5/rgs4), atypical mitochondrial subunits (cox4i2, higd1c, ndufa4l2) and potassium channels (task1, task3). The second aim of the project will be to assess whether developmental timing of Hif-2α activation affects the propensity to form paragangliomas in the fish, and whether the other candidate genes above modify the tumorigenic phenotypes. While we initially will focus on the neuroendocrine cell types known to be sensitive to oxygen, a long-term goal is to use the fish to also discover new and unexpected cell types that may harbour such sensitivity.

Translational potential
Identification of proteins underlying acute oxygen chemosensitivity will provide new therapeutic targets in pseudohypoxic tumours such as paraganglioma. While rare, these are notoriously difficult to treat, which will have a large impact upon the field. The mechanisms we discover may also provide therapeutic links to tumours that arise from other oxygen sensitive cell types such as small cell lung cancer.

Training opportunities
The student will join a vibrant group of researchers at Ludwig Cancer Research, and benefit from the combined expertise of the Ratcliffe and Bishop groups (oxygen sensing) and the White group (zebrafish genetics and cancer). Specific skills they will gain are *in vivo* imaging, creation of transgenic lines, biochemical analysis, and CRISPR manipulation (Cas9/Cas13).

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29. Exploiting synthetic defects in metabolism and DNA repair to improve the treatment of glioma and AML [1,2,3] - Prof. Peter McHugh

Primary Supervisor: Prof. Peter McHugh
Additional Supervisors: Prof. Chris Schofield
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Abstract

Current treatments for gliomas, an aggressive form of brain tumour, are non-specific and do not significantly increase clinical survival rates. Likewise, treatments for acute myeloid leukaemia (AML), especially relapsed disease, remain challenging. We have discovered a potential synthetic lethal response in tumours which exhibit a mutation in the isocitrate dehydrogenase (IDH) gene: IDH mutations are present in ~80% of gliomas and ~20% of AMLs. Specifically, the loss of several related DNA repair factors and IDH mutation leads to loss of cancer cell viability. Combining mechanistic cellular studies with our ongoing studies on small molecule DNA repair inhibitors could provide a route to treat these aggressive diseases.

Research objectives

Following the sequencing of thousands of glioblastoma samples, IDH was found to be mutated in in around 80% of tumours and in a significant subset of AML. A specific IDH1 (R132H) mutation occurs in about 70% of glioma tumours. IDH1 and IDH2 play key roles in redox metabolism, catalysing the oxidative decarboxylation of isocitrate to a-ketoglutarate (α-KG) and CO_2 producing NADPH (Cohen *et al.,* 2013). The cancer-associated mutant forms of IDH enzymes produce 2-hydroxyglutarate (2-HG), termed an '*oncometabolite*', a metabolic intermediate that helps tumour cells survive and proliferate. 2-HG acts as a competitive inhibitor of enzymes using a-ketoglutarate as a co-substrate, including a family of metal dependent dioxygenases, the ALKB family (Rohle *et al.,* 2013).

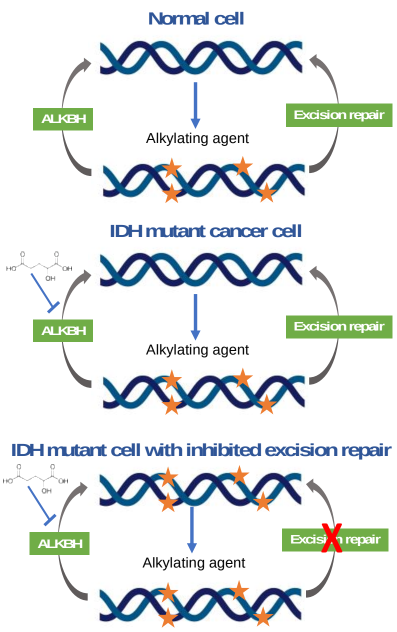

Figure 1. *Cells depend upon both the ALKBH enzymes and excision repair to remove DNA damage. Loss of both can be lethal in tumour cells.*

ALKBH2 and ALKBH3 are DNA repair enzymes that directly remove alkylation DNA damage. ALKBH2 is predominantly involved in repairing 1-methyladenine (1-meA) lesions on double-stranded DNA whilst a substrate for ALKBH3 is 3-methylcytosine (3-meC) lesions on single-stranded DNA (Dango *et al.,* 2011, Fedeles *et al.,* 2015).

Following a genetic screen we observed a synthetic lethal response to ALKBH2 and ALKBH3 loss that is induced by loss of several DNA repair excision repair factors. This, in turn, suggested that cancer cells harbouring IDH mutations could be sensitive to simultaneous loss of DNA exision repair genes by virtue of their reduced ALKB repair activity (**Fig. 1**). Follow-up studies suggest that this hypothesis is correct, and that inactivation of excision repair enzymes in IDH mutant tumour cells can be lethal.

We will explore the detailed biology of the synthetic relationship of DNA repair defects with the key clinically-relevant IDH1 R132H patient mutation, as well as ALKBH2/3 disrupted cells. By performing CRISPR-Cas9-based screens that target all know DNA damage response (DDR) genes in IDH mutant cells, we will survey and define the full range of DDR genes required for survival of IDH mutant cells and therefore idenfify additional new therapeutic targets. These findings will be validated in isogenic, matched glioblastoma and AML cell lines with and without IDH mutations, allowing us to explore this potential mechanism of synthetic lethality in a relevant cancer setting.

We will characterise the nature of the DNA repair defects observed in repair defective IDH mutant (and ALKBH deficient) cells using a wide range of well-established cellular, genetic and biochemical assays available to us. We will also define the pathway to cell death in cancer cells mutated in IDH1 or lacking ALKBH2/3 that is synthetic with additional repair pathway loss. Furthermore, inhibitors of IDH and ALKBH2/3 are available, developed in the group of Prof. Chris Schofield (Woon *et al.,* 2012). Molecules that target both wild-type IDH1 but also selectively inhibit the R132H form (as well as other clinically observed variants) have been developed and will be used to test our hypothesis that in IDH mutated cells can be killed through DNA repair pathway inhibition. Moreover, we will work in collaboration with our chemistry collaborators to generate improved inhibitors of key excision repair factors that can be used to selectively target IDH deficient tumours, work which builds upon well-developed work in this area in our laboratories. These tools, both genetic and chemical, will ultimately be combined by the student to perform key proof-of-principle experiments to explore this novel approach to treating two cancers of unmet need.

Translational potential
This proposal addresses a key priority of the Cancer Research UK and the Oxford Centre as it uses basic science to validate novel approaches to two difficult to treat cancers, AML and glioma.

Training opportunities
Cell culture, genomic engineering (CRISPR-Cas9 and base/prime editing), large-scale screens, general molecular biology methods, DNA damage and repair assays, advanced microscopy, cell sorting methods, protein purification chemical biology, protein science/enzyme inhibition, and biochemical assays. The student will also benefit from interactions with clinical colleagues involved in treating glioma and AML, as part of their thesis committee.

References:

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30. BLOod Test Trend for cancEr Detection (BLOTTED): an observational and prediction model development study using English primary care electronic health records data [1,2,3,4] – Dr. Pradeep S. Virdee

Primary Supervisor: Dr. Brian Nicholson
Additional Supervisors: Prof. Eva Morris
Eligibility: All tracks are eligible to apply for this project.

Abstract
Blood tests are commonly requested in NHS General Practice. Blood tests might be ordered when the patient attends their GP with symptoms or signs, to monitor a known medical condition, or as part of a "health check". Some clinical guidelines for GPs include recommendations that they should investigate for cancer if a blood test is lower or higher than a normal level. These recommendations are only helpful for a small number of cancers, such as bowel or pancreatic. Over time, a patient can build up a sequence, or trend, of blood test results. This trend might tell GPs more information than single blood test results. For example, a small drop from a steady trend could be more useful than waiting for the blood test to drop below a fixed level. However, the research has not yet been done to tell us which approach is most helpful to find patients who need referral to hospital for cancer investigation. In this research, we will explore if blood tests trend can detect patients with cancer better than single blood tests and check which patient groups trend is more helpful in and for what cancers, with primary focus on digestive and blood cancers.

Research Objectives

Background: A recent clinical review confirms that simple blood tests have an important role in identifying patients for cancer investigation [1]. However, analysis of National Cancer Diagnosis Audit in Primary Care data suggests that primary care investigations may delay referral [2]. Smarter use of blood tests to select patients for further cancer investigation could increase cancer yield and reduce unnecessary referrals. Our recent research highlighted that trends over time in serial blood tests could be more useful than single blood tests and non-specific symptoms to select patients for colorectal cancer investigation, with our colorectal cancer prediction models having good predictive ability [3,4]. However, trends are subtle so difficult to spot and may exist for various cancers.

Aim: To utilise trends in blood tests from primary care for early detection of cancer.

Objectives: There are three main objectives:

1) *identify trends among repeated blood tests indicative of cancer* – the student will learn of smoothing techniques, such as LOWESS, to graphically describe trends in each blood test, both overall and by personal, clinical, and cancer characteristics (e.g. age, sex, comorbidity, diagnosis route, site, stage). Collaborators: the Big Data Institute will collaborate on data curation and understanding of electronic health records data.

2) *assess predictive ability of blood test trends for different cancer types* – the student will learn of dynamic models, which utilise repeated measures data for assessing clinical outcomes. These include statistical models, such as joint modelling, and machine-learning models. Collaborators: the Computational Health Informatics Lab will collaborate on the machine-learning aspect for modelling trends and the Big Data Institute will collaborate on the interpretation of repeated measures data from national datasets.

3) *develop and test prediction models utilising blood test trend to optimise patient selection for referral* – the student will learn of the intricacies of developing and testing statistical and machine-learning prediction models and their clinical application. Collaborators: the Computational Health Informatics Lab will collaborate on the machine-learning aspect for developing prediction models and the Big Data Institute will collaborate on the interpretation of results from national datasets.

Data: Data from ~30 million patients from the CPRD primary care database is available to develop the models. It includes information on patient characteristics, deprivation, blood tests, symptoms, medications, cancer diagnosis, and other variables over 2000-2019. It is linked to the National Cancer Registration and Analysis Service, Hospital Episode Statistics databases, and Office of National Registration death database.

Outcomes: The main outcome will be prediction models that incorporate blood test trend for cancer risk. Outputs will include peer-reviewed journal publications for each objective separately and conference presentations.

Academic value: This research will develop an evidence base for blood test trend for cancer detection and inform clinical practice. The DPhil candidate will develop leadership and research skills in various areas, including primary care, electronic health records data, patient and public involvement, and more. The student will grow their academic publication record and research networks at courses and events. Collaborations in this research will provide direct access to further multidciplinary teams to improve efficiency in conducting this research.

Translational potential
The major route to a cancer diagnosis is GP-referral after the patient presents with symptoms. Despite millions of referrals each year, around half of cancer diagnoses in the UK are made late-stage, where the likelihood of survival is heavily reduced [5]. These prediction models have the potential to highlight high-risk patients before symptoms develop [6]. Initiating cancer investigation earlier could lead to the diagnosis of cancer at an earlier stage where likelihood of survival is increased. By helping GPs rule-in and rule-out patients for referral, cancer yield could be increased and unnecessary investigations reduced, minimising psychological and physical harm to patients and economic costs of unnecessary testing in the NHS.

Training opportunities
Throughout this DPhil, skills and experience will be developed in conducting independent research, working with routinely collected linked electronic health records data, patient and public involvement, statistical analysis, prediction modelling, general skill development, and more. The student will be offered a comprehensive training programme and encouraged to attend relevant courses.

Internal training: The Medical Sciences Division, Big Data Institute, and Richard Doll Building at the University of Oxford run extensive series of courses. Throughout the DPhil, the student will attend relevant courses, such as 'Conducting national and international research', 'Good Clinical Practice', 'An Introduction to Patient and Public Involvement', 'Statistical Data Analysis with R', 'Writing Skills', and 'Viva Preparation'. The student will be based in the Cancer Research Group, who will support the student and provide support throughout the research, and will also work closely with other multidisciplinary groups, such as the Medical Statistics Group and interdepartmental Computational Health Informatics Lab and Big Data Institute, to get direct additional support into various aspects of the DPhil, such as statistical methods and analystic software learning.

External training: External courses will cover general career development, research-specific training, and active collaboration building. These courses include 'Machine-Learning in R Software (Royal Statistical Society)', 'Analysis of Repeated Measures (Bristol)', 'Statistical Methods for Risk Prediction (Birmingham/Keele)', and 'Joint Modelling of Longitudinal and Survival Data (Leicester/Italy)', and CRUK DPhil courses/events. Attendence and presentation of findings at scientific conferences will also be encouraged.

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31. Tackling Cancers Defective of High-Fidelity DNA Repair Mechanisms [1,2,3,4] – Fumiko Esashi

Primary Supervisor: Fumiko Esashi
Additional Supervisors: Prof. Bass Hassan
Eligibility: All tracks are eligible to apply for this project.

Abstract
Recent technological advancements in cancer genomics have revealed significant cell-to-cell heterogeneity, highlighting the role of mutability in driving cancer evolution, posing therapeutic challenges (**1**). A recent study has indicated that the simultaneous impairment of two key high-fidelity DNA repair mechanisms, homologous recombination (HR) and mismatch repair (MMR), contributes to adaptive mutability and drug resistance (**2**). Notably, while HR loss is lethal in most cell types, MMR deficiency may mitigate this lethality. Our hypothesis is that MMR-defective backgrounds enable the survival and rapid evolution of cancer cells with HR loss. To investigate the impact of MMR/HR dual deficiencies on cancer development, we propose innovative experimental and bioinformatic approaches. Specifically, by conditionally inactivating HR in MMR-defective cellular model systems, we will identify genetic and genomic factors affecting cell survival. Additionally, we will perform association analyses based on somatic cancer mutations databases to uncover potential biomarkers and therapeutic strategies for early diagnosis and treatment of these cancers.

Research objectives
Individuals with inherited mutations within genes encoding MMR or HR factors exhibit increased risk to develop a wide range of cancers, as seen in patients with hereditary nonpolyposis colorectal cancer/Lynch syndrome (HNPCC/LS) or hereditary breast and ovarian cancer syndrome (HBOC), respectively. It is widely described that MMR defects confer mutator phenotypes with no lethal impact. Conversely, the biallelic mutations of genes encoding key HR regulators, such as the breast cancer susceptibility 2 (*BRCA2*) and the partner and localizer of BRCA2 (*PALB2*), elicits lethality, although monoallelic *BRCA2* or *PALB2* mutations are sufficient to increase cancer risk. Notably, a recent study suggests that the simultaneous impairment of MMR and HR drives adaptive mutability and drug resistance (**2**). However, the causal relationship of this phenomenon remains unclear. We hypothesise that MMR defective mutator background alleviates the lethal impact of HR loss and assists rapid evolution of cancer. This project tests this hypothesis and identifies genetic and genomic elements that are associated with MMR- and HR-defective cancers.

The genetic concept of 'synthetic lethality' or 'synthetic viability', involving the combination of mutations in multiple genes leading to cell death or growth, respectively, has gained rising attention in recent years for its potential for discovering new therapeutic targets in challenging cancers. Previous studies have relied on genome-wide loss-of-function screens in knockout cell lines. However, this approach has limitations, such as phenotypic changes obscured by secondary mutations. This project tackles these shortcomings by utilising the auxin-inducible degron (AID) technology (**3**) to conditionally deplete endogenous BRCA2 or PALB2 in MMR-defective HCT116 cell lines. This allows for highly-specific examination to uncover the direct impact of BRCA2 or PALB2 depletion in MMR-defective mutator background. Our preliminary study shows that, indeed, the acute depletion of BRCA2 or PALB2 confers lethality (**Fig 1A**), as well as increased sensitivity to a chemotherapeutic drug, poly (ADP-ribose) polymerase inhibitor olaparib (**Fig 1B, C**) as expected (**4**). To identify genes that affect normal survivals of BRCA2- or PALB2-depleted HCT116 cells, we will leverage the CRISPR-mediated modulation of transcription, namely **CRISPR interference/activation (CRISPRi/a)** (**5**). Our lab has already established the systems combining AID and CRISPRi/a. Using this technology, we will identify genetic factors, down- or up-regulation of which affects the survival of BRCA2- or PALB2-depleted cells.

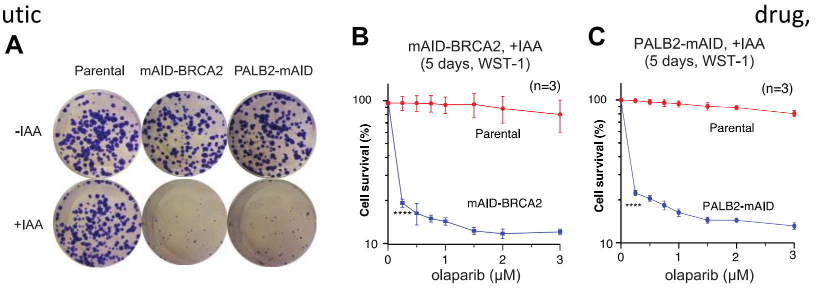

Figure 1. A. HCT116 mAID-BRCAs/PALB2-mAID cells were seeded in 6-well plates, and grown for 10 days with and without auxin (IAA). Colonies were then fixed and stained with crystal violet. **B-C:** HCT116 mAID-BRCA2/PALB2-mAID and parental HCT116 cells were first treated with IAA for 2 hours, and subsequently exposed to olaparib. After 5 days, cell survival was tested by WST assay. (n=3), error bars, SD. Asterisks indicate p value ≤ 0.0001 = ****.

In parallel, we will directly assess genome changes that occur upon BRCA2- or PALB2 depletion in HCT116 cells. We will isolate several clonal HCT116 cell lines which have survived upon depletion of BRCA2 or PALB2 for one month. Our preliminary analysis indicates distinct chromosomal aberrations in these cells, arising highly repetitive centromeric

regions of chromosomes. This observation is particularly intriguing as these repetitive regions are known to be targeted by MMR or HR (**6, 7**). To gain high resolution pictures of genome changes at these regions, we will conduct **long-read whole genome sequencing using Oxford Nanopore Technology (ONT)** that has advantages over traditional short-read sequencing. It enables the detection of alterations in repetitive sequences, as well as DNA modifications, such as CpG methylation. This approach is expected to provide a comprehensive understanding of the observed genome changes.
Finally, we will conduct **a bioinformatic assessment of publicly available somatic cancer mutation databases**, including COSMIC, to determine the prevalence of simultaneous impairment of MMR and HR pathways. We will initially focus colon cancers, which exhibit MMR deficiency in approximately 15% of cases. We will also explore the potential correlation between MMR/HR co-downregulation and the development of drug resistance. By examining the factors identified in our CRISPRi/a and long-read sequencing studies, we aim to uncover their association with drug resistance mechanisms. **This integrative approach will provide valuable insights into the underlying mechanisms driving drug resistance in these specific cancer types and inform the development of targeted therapeutic strategies.**

(ii) the collaborations involved and how these will be facilitated by the award:
The proposed project holds distinct opportunities to establish novel collaborations that will be facilitated by the award. This project stands out by venturing into new areas of research through long-read whole genome sequencing and cancer somatic mutation analysis, which have not been previously explored within our team nor in the field. The collaboration with **Dr Adam Cribbs** from the Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences (NDORMS) provides expertise in ONT-based long-read sequencing, offering new avenues for discovery and high-resolution analysis of genomic alterations associated with MMR and HR deficiencies. Dr. Cribbs' knowledge and resources in this field will significantly enhance the comprehensiveness of our research. Additionally, the second supervisor **Prof Bass Hassan**, a clinical research scientist at the Sir William Dunn School, brings expertise in cancer mutation analysis and perspectives for translational research. With Prof Hassan's guidance, we will navigate and interpret the vast dataset of cancer somatic mutations, gaining a deeper understanding of the genetic landscape and its implications for cancer development. These collaborations not only provide access to specialised techniques and resources in their respective expertise, but also open up new possibilities for uncovering critical insights into the genome instability, including those at repetitive regions, often found in difficult-to-treat cancers. Furthermore, their ability to bridge the gap between laboratory discoveries and clinical applications will significantly enhance the scientific impact and translational potential of our work. This interdisciplinary collaboration promotes knowledge exchange, fosters practical relevance, and ensures that our research has real-world implications in the fight against cancer.

Translational potential of the project.
The proposed project holds significant translational potential. Firstly, by identifying genetic and genomic elements that influence the survival of HR- and MMR-deficient hypermutable cells, our research is expected to identify early diagnostic markers and strategies for timely intervention. We can exploit vulnerabilities specific to these cancer cells, leading to more effective treatments while minimising adverse effects. Secondly, by integrating bioinformatic analyses of cancer genomes, this project is expected to reveal the prevalence of simultaneous impairments in MMR and HR pathways in colon cancer and potentially identify previously unspecified cancer 'signatures' associated with dual HR/MMR deficiency. Further assessment of correlation between MMR/HR co-downregulation and the development of drug resistance will offer an opportunity to develop novel therapeutic strategies for these challenging-to-treat cancers. In future, similar approach could be applied to assess other types of cancers, such as ovarian cancer and pancreatic cancer, which are commonly observed in HNPCC/LS and HBOC patients.

Training opportunities
Our research project offers valuable training opportunities in key areas of cancer research, including: (1) cell culture techniques, encompassing cell line maintenance, manipulation, and experimental assays; (2) the opportunity to learn and apply long-read sequencing techniques, including sample preparation, data generation, and analysis; and (3) bioinformatic techniques for analysing publicly available somatic cancer mutation databases and exploring genetic patterns. By providing training in these areas, our project equips researchers with essential skills for future scientific endeavors in the field of cancer research. The candidates will be well supported in the Dunn school in related methods training, including CRISPR, light microscopy imaging and flow cytometry through in-house facilities, namely the Genome Engineering Oxford (led by Dr Joey Riepsaame), the Dunn School Bioimaging Facility (led by Dr Alan Wainman), and the Don Mason Facility of Flow Cytometry (led by Dr Robert Hedley), respectively.

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32. Predicting Response to Therapy in Oesophageal Cancer [4] – Prof. Jens Rittscher

Primary Supervisor: Prof. Jens Rittscher
Additional Supervisors: Elizabeth Smyth
Eligibility: Track 4 students are eligible to apply for this project.

Abstract
The incidence of oesophageal adenocarcinoma (OAC) has increased over the last three decades. Approximately 8500 new cases of OAC are diagnosed every year in the UK. Only one in seven patients survive for more than 5 years. OAC is a cancer that is associated with extensive treatment requirements and in consequence a considerable decline in health-related quality of life. Curative treatment includes a combination of chemotherapy, radiotherapy, and/or immunotherapy followed by extensive surgery, however less than half of patients are cured using this approach.

Research objectives
The goal of this project is the development of tissue-based biomarkers that can accurately predict therapy response to save time, improve quality of life, and potentially improve survival. Here, we will be building on our recent work on an AI based approach for predicting response to radiotherapy in colorectal cancer on the basis of digitised H&E histology images [1]. Enhancing the interpretability of AI model predictions in such a way that they provide contextual information that effectively supports clinical decision making is an important aspect which we aim to refine and extend. Morpho-molecular correlates [2] that relate morphological features with disease relevant molecular traits are core to generating interpretable contextual information. The main aims of this projects are:

- Predicting which patients benefit from radiotherapy or chemotherapy,
- Predicting response to immune checkpoint inhibitors,
- Develop histology-based biomarkers to enable precision oncology.

Training Opportunities
This project provides the opportunity of developing state-of-art deep learning methods in an interdisciplinary setting. You will be working with oncologists, pathologists, and engineers to formulate the clinical questions and translate these into technical requirements. To be successful in this project you will have a background in machine learning and a passion for developing translational AI solutions in a responsible manner. In addition to developing the underlying methodology, you will assess the inherent bias of specific clinical trial cohorts, and develop validation strategies that ensure robustness. As part of the development of morpho-molecular correlates you will perform a multi-omic analysis of existing patient cohorts.

Dataset	Type	What is available
VESTIGE	Phase II randomised clinical trial Adjuvant chemo vs immunotherapy in high risk (after neoadjuvant chemo)	Biopsy for some patient Resection for most Tumour response grading (PDL1, MMR, planned: Claudin 18.2, HER2, FGFR2, RNAseq)
OCCAMS Cambridge based	Prospective UK dataset Chemotherapy plus surgery outcomes	WGS RNAseq for 250 Methylation

	Limited radiotherapy	IHC lacking but could be done
GSTT/RMH dataset	Prospective UK dataset Chemotherapy and surgery oucomes Limited radiotherapy	No omics Tumour response grading

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33. Developing single-cell transcriptomics tools for PARP inhibitor resistance in BRCA1/2-deficient cells and tumours[1,2,3,4] – Prof Madelena Tarsounas

Primary Supervisor: Prof Madelena Tarsounas
Additional Supervisors: Dr. Christiana Kartsonaki
Eligibility: All tracks are eligible to apply for this project.

Abstract

Cells and tumours with compromised breast cancer susceptibility genes *BRCA1* or *BRCA2* retain the ability to proliferate, in spite of the severe genomic instability caused by accumulation of DNA lesions. This vulnerability is exploited by highly-specific therapies that enhance the susceptibility of *BRCA1/2*-deficient tumours to DNA damaging agents, with the poly-ADP ribose polymerase inhibitors (PARPi; e.g. olaparib) as a prominent example. In spite of clear therapeutic benefits, cure rates for *BRCA1/2*-mutated cancers remain low, as patients frequently develop resistance to PARPi. Several mechanisms of PARPi resistance have been reported. However, PARPi resistance remains a critical problem in the clinic, limiting sustained responses to these drugs. Here, we aim to identify transcriptional signatures associated with PARPi resistance, specifically olaparib resistance.

To identify such signatures, we will perform single-cell RNA sequencing (scRNAseq) using *BRCA1/2*-deficient cells in culture and cells obtained from patient-derived xenograft (PDX) models that have acquired olaparib-resistance upon prolonged exposure to olaparib. We will furthermore combine scRNAseq and EdUseq data to test whether replication failure at specific genomic sites could interfere with transcription and therefore represent the underlying mechanism of the identified transcriptome alterations.

In the longer term, this line of research can lead to predictive markers for patient response to olaparib, which could facilitate early patient stratification and the development of personalized treatment strategies.

Research Objectives

The work proposed here will help elucidate, at single-cell resolution, the relationship between the emergence of rare, tumour-initiating cells sub-populations within tumours lacking *BRCA1* or *BRCA2* genes and the response to the PARP inhibitor olaparib. Accordingly, we will pursue two main objectives:

a. Characterize the single-cell transcriptomic landscape of olaparib-resistant *BRCA1/2*-deficient cells in culture. The scRNAseq technology will enable us to generate gene expression profiles of single cells and to identify cell sub-populations with specific transcriptional signatures. To achieve this first objective, scRNAseq will be carried out in populations of olaparib-sensitive and -resistant *BRCA1/2*-deficient cells, already generated in Tarsounas lab. We will prepare libraries from each cell line, before and after olaparib resistance onset, to sequence between 7,000 and 10,000 cells using the standard protocol of the Chromium Single-Cell 3' gene expression profiling solution (10x Genomics).

Unsupervised clustering approaches will be developed to classify cells into sub-groups with specific signatures (e.g. immune response, metastasis etc.) and to monitor cell dynamics using algorithms for pseudotime analysis. We will apply this combination of analytical approaches to the cell lines that are sensitive or become resistant to Olaparib, and anticipate that this will allow us to identify cell clusters with unique patterns of gene expression, which could not be resolved at the whole-cell population level. In addition, this approach will enable us to identify eventual differences between the signatures specific to *BRCA1*- and *BRCA2*-deficient cells. Lastly, the collection of signatures identified for distinct cell subpopulations selected by olaparib will be further explored in the large METABRIC and TCGA PanCancer Atlas breast and ovarian cancer cohorts (5,098 samples, among which 355 and 362 carry alterations in *BRCA1* and *BRCA2*, respectively), specifically to assess their prognostic ability through univariable and multivariable regression models.

b. Characterize the single-cell transcriptomic landscape of olaparib-resistant *BRCA1/2*-deficient PDX tumours *in vivo*. In addition to linking the transcriptomic signatures of olaparib-resistant cell subpopulations to tumour gene expression data and clinical information found in databases (e.g. TCGA, METABRIC), we will recapitulate *in vivo* the results obtained *in vitro* using cell cultures. To achieve this, scRNA-seq will be carried out in cell suspensions prepared from *BRCA1*- or *BRCA2*-mutated (n = 3 $BRCA1^{-/-}$ and n = 1 $BRCA2^{-/-}$) olaparib-naïve and -resistant patient-derived xenografts (PDX). In these models resistance emerged after treatment with olaparib for up to 150 days, when individual tumours regrew. These models are also already available for processing in Tarsounas lab.

(ii) This project will facilitate the collaboration between basic cell biology and bioinformatics, and the student funded here will be trained in and benefit from both types of expertise. Importantly, this collaboration will enable the co-applicant, Dr Christiana Kartsonaki, who is an early career researcher within the NDPH, to achieve scientific independence. Securing this CRUK award will enable her to co-supervise a graduate student in a new area of research, and at the same time, will place her in a stronger position for further funding applications.

Translational potential of the project.

In spite of initial responses to targeted therapies such as PARPi, *BRCA1/2*-deficient tumours develop a resistance to these therapies. PARPi resistance often entails genomic rearrangements and mutations that trigger rewiring of the damage response pathways within the tumour so that apoptotic responses to treatment are replaced by cell survival and metastasis. Here we anticipate to identify new, robust transcriptional signatures associated with Olaparib resistance, which can be used to stratify patients for PARPi therapy. In addition, these gene expression profiles will identify vulnerabilities that can be exploited to target resistant disease. In the longer term, these approaches can be used to develop patient screening protocols using machine learning and statistical methods.

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34. Harnessing measurements of the tumour microenvironment to improve the early detection of prostate cancer. [1,3,4] – Professor Richard Bryant

Primary Supervisor: Professor Richard Bryant
Additional Supervisors: Dr James Grist
Eligibility: Track 1, 3 and 4 students are eligible to apply for this project.

Abstract
Approximately 190,000 invasive prostate biopsy procedures are performed each year in the UK alone. The current standard of care recommends the use of pre-biopsy MRI scan to improve the accuracy of the prostate biopsy. Despite the success of using the '*Prostate Imaging Reporting And Data System'* (PI-RADS) (1) score (and similar scores such as the Likert score) in the reporting of MRI scan images ahead of targeted prostate biopsy, around 5-15% of clinically significant cancers would potentially be missed if individuals with an elevated age-specific PSA and normal clinical imaging did not receive a prostate biopsy. It is difficult to derive precise figures, but this would likely amount to several thousand men with a delayed or missed diagnosis of clinically significant prostate cancer each year in the UK alone, the clinical impact of which is currently unknown.

Whilst changes in imaging contrast due to tumour growth tend to be slow, primarily due to the inherently low sensitivity of clinical MRI, alterations in the motion of water (due to the restriction of motion by highly cellular regions of cancerous tissue) and the direct metabolic reprograming of cancerous cells to upregulate production of lactate to fuel proliferation (known as the "Warburg Effect") can be harnessed to potentially provide early detection of prostate cancer (2). Indeed, the first in-human trial using hyperpolarised MRI demonstrated the ability of this technique to detect oncological metabolism in the absence of conventional imaging alterations (see Figure 1 showing cancerous metabolism in both the left and right sides of the prostate, with conventional imaging showing changes only on the right side), and Restricted Spectrum Imaging (RSI) has been shown to be highly sensitivity in the detection of prostate cancer (3).

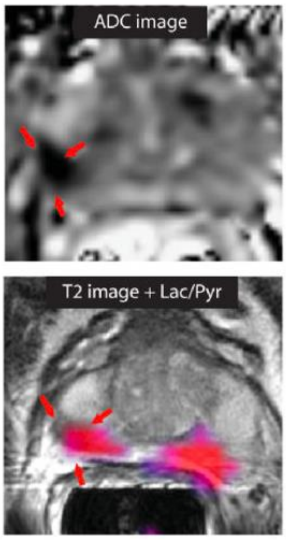

Figure 3

We have experience in advanced imaging techniques to study neurological and cardiovascular disease, and now is an opportune time to expand these techniques to benefit the oncological arena, this being an area of important unmet clinical need for prostate cancer. The DPhil student will work with the surgical and radiological teams to recruit and image a cohort of 40 men aged between 60-69 years (as defined by power calculations from previous studies in Oxford) being investigated for possible prostate cancer. Participants will receive standard of care pre-biopsy MRI imaging, with the functional MRI appended to the end of the clinical session. The biopsy process will be performed based on the clinical imaging, as per our current practice. Of the N = 40 men in this pilot study, 10 individuals will have a normal (PIRADS 1-2) pre-biopsy MRI, 10 individuals will have an equivocal (PIRADS 3) MRI, and 20 will have a 'suspicious' (PIRADS 4-5) pre-biopsy MRI. The inclusion of patients with either a PIRADS 1-2 or a PIRADS 3 MRI is important given that individuals in the age range of 60-69 years often have diffuse PIRADS 3 change within the peripheral zone of the prostate gland due to their young age. All 40 individuals will undergo prostate biopsy following the performance of the clinical and functional scans, with targeted and systematic biopsy cores being obtained for the N = 30 men with clinical lesions (PIRADS 3-5), and systematic biopsies alone being obtained for men with a normal (PIRADS 1-2) clinical imaging, as per our current protocol. The clinical and functional MR images will then be correlated with the final prostate biopsy pathology, to test the hypothesis that some clinically significant prostate cancer cases may solely be visible on functional MRI and not on standard clinical imaging. This approach will improve sensitivity of detection – with RSI acquisitions, if shown to be beneficial in the detection of prostate cancer, ready to be run in clinical practice in every patient in our NHS trust, and beyond, and hyperpolarised MRI offered as a clinical scan in targeted patients at a later time point (2-5 years). In turn, this will benefit many men being investigated for possible prostate cancer using MRI scans as part of the clinical pathway.

We currently have an ethics application going through internal review to enable this project. See figure 2 for the workflow for this project.

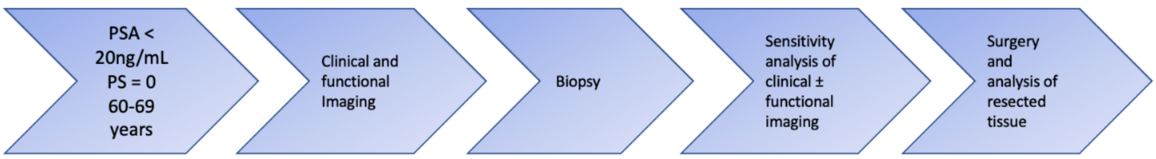

Figure 4

Research objectives

The two key objectives for this DPhil project are:

1) To establish repeatable imaging protocols for assessing functional and clinical MRI within the prostate. A sub-cohort (N = 20, 5 from each PI-RADS group) of patients and healthy controls (N = 8) will undergo test re-test imaging on the same day to assess for the repeatability of imaging results. **Collaborators required:** Dr James Grist, Dr Ruth MacPherson, Professor Damian Tyler. **Outcome:** Presentation of results at leading radiological conferences (European Congress of Radiology, International Society of Magnetic Resonance in Medicine), and publication of results in a leading medical imaging journal.

2) To perform a clinical study assessing the added value of functional MRI to the routine clinical protocol. The data from the full cohort of patients will be analysed to assess for the sensitivity and specificity of the clinical, functional, and combined imaging approaches for the detection of biopsy proven clinically significant prostate cancer. **Collaborators required:** Dr James Grist, Dr Ruth MacPherson, Dr Richard Colling, and Professor Ian Mills. **Outcome:** Presentation of results at a leading radiological conference and publication of results in a leading medical journal.

Translational potential

If successful, this project will enable the early detection of clinically significant prostate cancer in a cohort of patients undergoing investigation for possible prostate cancer, who might otherwise have a delayed or missed diagnosis. However, the further clinical translation and impact of this project will be felt from the incorporation of these additional functional MRI approaches into clinical practice in Oxford and beyond – thus benefitting a larger population of patients with suspected prostate cancer. However, beyond this impact there is also the potential to apply these advanced imaging methods to other cancers, to detect early therapeutic response to chemo/radiotherapy – for example paediatric brain tumours, renal cancer, and breast cancer.

Training opportunities

The student will be provided with training in clinical research and patient recruitment by Professor Richard Bryant, and will be provided with training from Professor Damian Tyler and Dr James Grist in MRI physics, data acquisition, image reconstruction and quantitative post-processing for both hyperpolarised MRI and RSI. Further training in image co-registration will be provided. Dr Ruth MacPherson will provide training in clinical image analysis and interpretation, and Dr Richard Colling will provide training in histology and will work with Professor Ian Mills to complement this with molecular phenotyping of cancerous tissue. By the end of the project, the student will have a wide-ranging skill set that will be of great benefit to further their career in clinical research.

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35. Investigating Hypoxic Adaptation in Glioblastoma (GBM) Stem Cells through Pooled Kinome-Wide CRISPR-Cas9 Knockout Screen [1,2,3] – Dr. Sneha Anand

Primary Supervisor: Dr. Sneha Anand
Additional Supervisors: Prof. Daniel Ebner
Eligibility: Track 3 students are eligible to apply for this project.

Abstract

Glioblastoma (GBM) is a highly aggressive brain tumour characterized by its resistance to treatment and invasive nature. Central to this resistance is the existence and chemo-radiotherapeutic resistance of glioblastoma stem cells (GSC) within a hypoxic core of the tumour. Hypoxia, a condition of low oxygen availability, is a critical feature of the tumor microenvironment (TME) that promotes GBM progression, migration, immune evasion, and therapy resistance and could be a major factor in the generation of recurrent GBM which leads to poor survival outcomes of GBM patients. Protein kinases, involved in various signalling pathways, have been implicated in the response of cancer cells to hypoxia (Astrid A Glück 2015). They are attractive targets for cancer therapy due to their critical roles in oncogenic processes and the availability of kinase inhibitors. In the context of hypoxia, molecular crosstalk between Hypoxia Inducible factors (HIFs) and protein kinases plays a crucial role in modulating cellular responses to low oxygen levels. Dysregulated kinase activities are common in cancer, and hypoxia can further disrupt the cellular kinome network, enhancing the robustness of oncogenic pathways. Targeting hypoxia-dependent kinase signalling hubs holds the potential for attenuating the survival advantages conferred by hypoxic adaptation. In this research proposal, we aim to investigate the molecular basis underlying the adaptation and survival of GSC cells in hypoxia. We propose to conduct a pooled kinome-wide CRISPR-Cas9 knockout screen using a lentiviral single guide RNA (sgRNA) library that targets human kinases (John G Doench 2016). The goal is to identify key kinases that contribute to the hypoxic adaptation of GBM cells, potentially uncovering new therapeutic targets for GBM treatment.

Research objectives

This research project aims to investigate the molecular mechanisms underlying hypoxic adaptation in GBM using patient-derived GSC lines. GSCs are known to play a significant role in tumor initiation and recurrence, and hypoxia promotes their self-renewal and invasive properties. The study involves characterizing GSC behaviour in low oxygen conditions through hypoxia chamber culture, hypoxia marker staining, and analysis of hypoxia-inducible factor (HIF) expression using various techniques such as immunofluorescence staining, western blotting, or quantitative PCR. Following characterization, a kinome-wide CRISPR-Cas9 knockout screen (Fig.1) will be performed using a lentiviral sgRNA library targeting human kinases. The abundance of sgRNAs targeting kinases will be analysed through next-generation sequencing, and bioinformatics analysis will be conducted to identify kinases exhibiting differential knockout effects in hypoxic compared to normoxic conditions. The identified kinases will undergo functional characterization to understand their specific roles in hypoxic adaptation. Ultimately, the study aims to uncover potential therapeutic targets for disrupting hypoxia-dependent mechanisms in GBM.

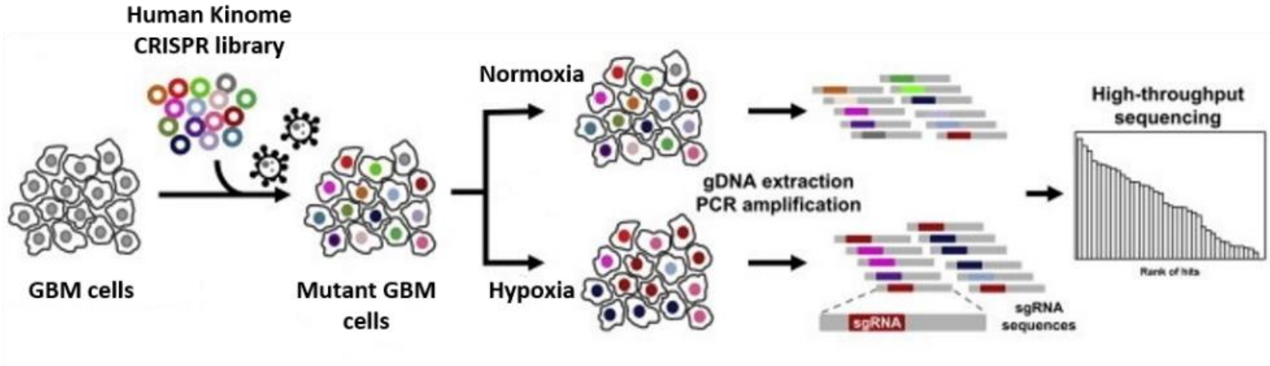

Figure 1: Schematic illustrating the CRISPR knockout screen: Lentiviral transduction of patient-derived GSC lines with the sgRNA library to induce kinase knockout. Following this, cells will be divided into normoxia and hypoxia groups and maintained in their respective conditions for a specific duration. Genomic DNA extraction from cells in both normoxia and hypoxia groups. Next-generation sequencing to analyze the abundance of sgRNAs targeting kinases. Data processing to

determine sgRNA frequency and identify kinases significantly affected in hypoxic conditions compared to normoxic conditions.

Expected Outcomes:

1. Characterization of GSC Behaviour in Hypoxic Conditions: The study will provide insights into the behaviour of GSCs under hypoxic conditions. This includes understanding the activation of specific signalling pathways in response to low oxygen levels, such as the upregulation of hypoxia-inducible factors (HIFs) and their downstream target genes related to angiogenesis, glycolytic metabolism, and therapy resistance.
2. Identification of Hypoxia-Related Therapeutic Targets: The characterization of GSC behaviour in hypoxic conditions can lead to the identification of potential therapeutic targets specifically active in the hypoxic microenvironment. These targets may offer opportunities for the development of novel therapies aimed at disrupting hypoxia-mediated adaptations and improving treatment outcomes for glioblastoma patients.
3. Identification of Hypoxia-Responsive Kinases: Through the kinome-wide CRISPR-Cas9 knockout screen, the study will identify kinases that play a critical role in GBM cell survival and adaptation in hypoxia. This information can provide valuable insights into the molecular mechanisms underlying hypoxic adaptation in GBM.
4. Potential Therapeutic Targets: The study will yield a list of kinases whose inhibition could potentially attenuate the survival advantages conferred by hypoxia in GBM. These kinases may serve as promising targets for the development of novel anti-GBM therapies, with the aim of enhancing treatment efficacy and overcoming drug resistance associated with hypoxia.
5. Manuscript Generation: The research project aims to generate two manuscripts from the study, highlighting the findings, methodologies, and implications of the research. These manuscripts will contribute to the scientific literature and would benefit other researchers and clinicians in the field.

Translational Potential

Understanding the molecular basis of GBM cell adaptation to hypoxia is crucial for developing effective treatment strategies against this aggressive brain tumor. The identified kinases can be further investigated for their roles in hypoxia-mediated processes, such as angiogenesis, metabolism, and immune evasion. Additionally, functional validation studies can be conducted to confirm the impact of specific kinases on GBM cell behavior under hypoxic conditions.

Overall, the findings from this study will contribute to a deeper understanding of GBM biology and provide potential targets for the development of novel therapeutic strategies to improve patient outcomes in GBM treatment.

Training opportunities

This research project offers valuable training opportunities in CRISPR-based cell screening, cell biology, molecular biology, and bioinformatics analysis. The student will learn essential techniques for gene editing, high-throughput screening, and cellular manipulation. They will gain hands-on experience in cell culture, molecular biology techniques, and an introduction to bioinformatics data analysis. The project will enhance their skills in experimental design, data interpretation, literature review, and scientific writing. Overall, this project provides a solid foundation for their DPhil studies and future research in cancer biology and therapeutics.

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36. Dietary modification to augment colorectal cancer treatment [1,2,3] – Dr Dimitrios Koutoukidis

Primary Supervisor: Dr Dimitrios Koutoukidis
Additional Supervisors: Prof. Simon Buczacki and Dr Victoria Woodcock
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Abstract
Obesity is associated with worse progression-free survival among cancer survivors. One of the reasons might be that patients with obesity are receiving lower doses of chemotherapy compared with patients without obesity. Preclinical models show that energy restriction reduces chemotherapy toxicity and may augment chemotherapy efficacy. This is hypothesised to be driven by improvements in glucose regulation and reductions in IGF-1 through stress resistance in healthy cells and stress sensitisation in cancer cells. Furthermore, they suggest that standalone energy restriction is not as efficacious as a combination of energy restriction and established cancer treatments, such as chemotherapy.[1]

Although fasting itself around chemotherapy seems to not raise safety concerns based on a limited number of clinical cases and has shown some preliminary potential for increasing chemotherapy efficacy (higher radiological response and percentage of Miller Payne 4/5), adherence is challenging, the employed protocols vary, and the substantial increase in energy intake for 2-3-week period following the short-term (24-60h) fasting period during chemotherapy administration may negate its benefits. Furthermore, the 24-60h fasting that has shown benefit in mice cannot be expected to have the same effect as a 24-60h fast in humans, given the different physiology and lifespan. Instead, it is likely that a longer period of energy restriction will be required to achieve results of similar magnitude. Additionally, intentional weight loss may be considered counterintuitive during cancer treatment as weight loss, primarily muscle mass loss, has been associated with worse outcomes.[2 3]

Existing low-energy dietary interventions in other clinical settings have been shown to affect the postulated mechanism. Intentional weight loss among people with obesity in other clinical settings has reliably improved glucose regulation and reduced insulin with the relationship following a dose-response manner and substantial weight loss to be required for a clinically meaningful improvement in glucose regulation to be observed. We and others have shown high adherence and significant weight loss with 800kcal/day diets including high-protein nutritionally replete total diet replacement with soups and shakes and food-based low-carbohydrate diets.[4-7]

This DPhil project will aim to adapt one such existing intervention, test its feasibility in a randomised controlled trial, and explore the mechanisms through which the intervention may increase chemotherapy efficacy.

Research objectives
WP1: Adapting the intervention to the setting.
Using the established Intervention Mapping approach and the MRC framework for complex interventions, together with involvement of relevant patient representatives, clinicians, and researchers. Through an iterative process, WP1 will focus on adapting an existing feasible and effective total diet replacement intervention.

WP2: Testing the feasibility of the intervention
WP2 will involve running a randomised controlled trial to test the feasibility of the intervention. Specifically, the feasibility will be judged against pre-determined criteria on

- recruitment
- adherence to the diet
- retention at the last follow-up
- experience of participants following the intervention.

<u>Population</u>: Patients with obesity (BMI ≥ 30kg/m^2) and metastatic colorectal cancer.
<u>Intervention</u>: The exact nature of the intervention will depend on the findings on WP1, but we envisage that it involve a low-energy (800kcal/day) intervention through total diet replacement or through food-based low-carb diet from 5 days before each chemotherapy cycle to 1 day after each cycle followed by advice for a healthy balanced diet for the remaining period.

Figure 1: Proposed diet for the first 2 cycles of chemotherapy by day. Green indicates low-energy diet Orange indicates healthy balanced diet, and blue indicates days of chemotherapy.

Days	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
	Low-energy diet								Healthy balanced diet						Low-energy diet								Healthy balanced diet					
						1st cycle														2nd cycle								

<u>Comparator</u>: Standard care (chemotherapy and existing CRUK leaflet on diet during treatment).
<u>Sample size</u>: With 72 patients (36 in each group), the trial will be 90% powered at one-sided 5% level based on the normal approximation approach to detect whether the proportions for adherence and retention criteria are truly above 35% and 65%, respectively, based on an alternative hypothesis that they will be above 60% and 85%, respectively.
<u>Feasibility outcomes</u>: These will include rates of recruitment, adherence, and retention.
<u>Process outcomes</u>: Experience and acceptability of the intervention based on semi-structured qualitative interviews with intervention participants.
<u>Exploratory outcomes</u>: Relative dose intensity, toxicity, and tolerance; and health-related quality of life.

WP3: Exploring the mechanisms of the intervention
The objectives of this WP is to explore potential mechanisms through which the intervention may increase chemotherapy efficacy.
<u>Process</u>: Participants will be asked to provide blood and urine samples before and after each cycle of the low-energy diet and each cycle of chemotherapy. Samples will be analysed for

- inflammation and immune serum markers (using the Olink Explore384 and macc cytometry (CyTOF)). The Olink panel will enable quantification of 368 low-abundance inflammatory cytokines and soluble immune mediators and CyTOF will enable quantification and functional assessment of >50 distinct immune cell subsets.
- urine and serum metabolomics and proteomicsmTOR & pmTOR PBMC ELISA.

Translational potential
Developing novel therapeutics that are safe and effective with minimal side effects is a key priority for both cancer research and patient care. This need is highest among patients with metastatic disease, which this DPhil targets. This project will pave the way for a definitive clinical trial that will assess the effectiveness of this intervention in improving response to chemotherapy. If successful, this is likely to reduce the burden for patients and increase their progression-free survival.

Training opportunities
The student will have the opportunity to be trained in an array of different methodologies, including the development of complex interventions, recruiting to, managing, and analysis the results of a randomised clinical trial, qualitative analysis of interviews, laboratory techniques, and analysing of complex large-scale biomarker data. This will include targeted formal courses within and outside Oxford as well as on-the-job training. They will have access to standard operating procedures from a relevant clinical trials unit, weekly meetings with supervisors, and access to a diverse pool of researchers applying the above methodologies at both NDPCHS and NDS.

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37. Investigating the adaptive immune responses to tumour neo-antigen and the impact on patient disease course. [1,2,3,4] –Assoc Prof. Rachael Bashford-Rogers

Primary Supervisor: Assoc Prof. Rachael Bashford-Rogers
Additional Supervisors: Dr. Isabela Pedroza-Pacheco
Eligibility: All tracks are eligible to apply for this project.

Abstract

B and T cell infiltration has prognostic significance in solid tumours, and ongoing studies are investigating their phenotypes through single cell transcriptomics and spatial imaging. Characterising the B and T cell response to tumour cells, particularly their antigenic specificities, will be key to developing more immunologically appropriate cancer therapies. Here we propose a DPhil studentship project to develop novel technologies to be able to bridge the gap between B and T cell population phenotypes, migration, and antibody/TCR reactivity, thus giving a unique perspective on the development of anti-self, anti-tumour and anti-non-self-responses. **Therefore, this project will characterise the nature, function and migration of myeloid cells in primary and metastatic tumours, blood and lymph nodes to help direct us towards understanding and potentially modulating these cells for more targeted therapies. This project will involve the development a novel high-throughput method to probe the antigen specificities of B and T cells, which will be coupled with single cell resolution of clonal phenotype and single cell transcriptome. This will be used to investigate the development and role of tumour-infiltrating B and T cells across a range of tumours with varying degrees of immunogenicity.** Single-cell RNA sequencing has emerged as a powerful tool to investigate cell-to-cell variation (Buettner et al., 2015). Having expertise in developing both novel experimental and computational methodologies for understanding immune-cell diversity and dynamics (Bashford-Rogers et al., 2019), as well as disease-specific pathology and genetics, we are in a unique position to combine single-cell RNA sequencing, clonal tracking through the BCR/TCR, and functional experiments.

Research objectives

This project aims to investigate the B and T cell immune response of circulating and tumour-infiltrating B cells across a range of tumours with different levels of immunogenicity and neo-antigen expression, including pancreatic and renal cancers. This will involve the development of a novel platform to answer key questions in the field of tumour immunology including:

- Defining whether B and T cell migration to lymph nodes reflect the response within the primary tumour.
- Determining if tumour-associated B cell subsets produce antibodies against self- or non-self antigen, poly-reactive, or highly specific to tumour cells, and how does tumour cell neo-antigen variation and expression level dictate antibody response and protection.
- Determining the balance of whether B and T cells serve a pro- or an anti-tumourigenic function.
- Investigating the spatial distribution of T and B cell anti-tumour clones within tumour tissue and determine their functional role during tumour immunosurveillance.
- Characterising the molecular mechanisms defining pancreatic B and T cell activation and regulation.
- Building computational models of immunosurveillance of tumours to help stratify patients for therapies and predict outcome.

Translational potential

This study will provide a unique platform to understand the probe between tumour neo-antigen, B and T cell immune-surveillance and specificity, and B and T cell phenotype, with the overall aim of highlighting improved therapeutic options and patient outcomes. Furthermore, the methods developed here will not just be broadly applicable to cancer, but will have wider applications in immunology and biotechnology. This will be achieved through the development and application of novel experimental and computational approaches, working in partnership with a global network of clinicians, immunologists and sample cohorts.

Training opportunities

The DPhil will gain experience and training in laboratory molecular biology and single cell methods, bioinformatics and immunology. These include:

- Cell sorting and single-cell RNA sequencing of patient samples.
- Development of novel experimental and computational pipelines for the analysis of novel single-cell datasets.
- Validation of associations using a wide range of immunological techniques.
- The project will work in partnership with a global network of clinicians, immunologists and sample cohorts.

The Bashford-Rogers laboratory has a strong track record of collaboration over the last 15 years and established systems for co-supervision. The postdoctoral fellows and the PIs will provide daily support and supervision when needed. The student will learn established protocols for imaging, advanced sequencing, and genomic analysis from existing postdoctoral and DPhil fellows. The candidate will be expected to participate in the weekly laboratory meetings and encouraged to present research at national and international meetings.

References

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(2) Activated regulatory T-cells, dysfunctional and senescent T-cells hinder the immunity in pancreatic cancer *(Cancers, 2021)* Shivan Sivakumar, Enas Abu-Shah, David Ahern, Edward H Arbe-Barnes, Nagina Mangal, Srikanth Reddy, Aniko Rendek, Alistair Easton, Elke Kurz, Michael Silva, Lara R Heij, Zahir Soonawalla, Rachael Bashford-Rogers, Mark R Middleton, Michael Dustin

(3) Shared D-J rearrangements reveal cell of origin of TCF3-ZNF384 and PTPN11 mutations in monozygotic twins with concordant BCP-ALL (*Blood, 2020*) Clara Bueno, Paola Ballerini, Ignacio Varela, Pablo Menendez, Rachael Bashford-Rogers

(4) Analysis of the B cell receptor repertoire in six immune-mediated diseases. (*Nature, 2019*) RJM Bashford-Rogers, L Bergamaschi, EF McKinney, DC Pombal, F Mescia, JC Lee, DC Thomas, SM Flint, P Kellam, DRW Jayne, PA Lyons, KGC Smith

(5) The integrated genomic and immune landscapes of lethal metastatic breast cancer. (*Cell Reports*, 2019) Leticia De Mattos-Arruda, Stephen-John Sammut, Edith M. Ross, Rachael Bashford-Rogers, Erez Greenstein, Havell Markus,Sandro Morganella, Yvonne Teng, Yosef Maruvka, Bernard Pereira, Oscar Rueda, Suet-Feung Chin, Tania Contente-Cuomo, Regina Mayor, Alexandra Arias, Raza Ali, Wei Cope, Daniel Tiezzi, Dan Reshef,Elena Martinez, Vicente Peg, Santiago Ramon y Cajal, Javier Cortes, George Vassiliou, Gad Getz, Serena Nik-Zainal, Muhammed Murtaza, Nir Friedman, Florian Markowetz, Joan Seoane and Carlos Caldas

38. Epigenetic control of cancer cell phenotypes via nuclear F-actin based chromosome motility. [1,2,3] – Prof. Eric O'Neil

Primary Supervisor: Prof. Eric O'Neil
Additional Supervisors: Prof. Yang Shi
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Abstract
The hippo tumour suppressor pathway regulates tissue size in development and although the contribution of this pathway to cancer is evident from tumour models and pan-cancer transcriptomics, somatic mutations are rare[4]. Our research has demonstrated how epigenetic silencing of RASSF1A is responsible for YAP activation in human tumours and correlates with poor survival across all major solid malignancies. Such 'epigenetic plasticity' allows dynamic switching between phenotypes and supports progression of lesions and the appearance of cancer stem-like cells (CSCs) in solid tumours[42]. During development, increasing evidence implicates the co-factor YAP1 as a key determinant of phenotype by supporting pluripotency or differentiation through activation of distinct transcription programmes in response to RASSF1-hippo signalling[5]. Moreover, the hippo pathway transduces mechanical forces from the microenvironment to guide proliferation, stem cell behaviour and differentiation[6]. Our recent work has identified RASSF1A and MST2 reside at the nuclear envelope to sense mechanical force and influence both chromatin and nuclear actin. This project aims to consolidate these discoveries to understand how genomic motility between repressive heterochromatin and phase separated transcription factories are controlled by nuclear actin to influence cell phenotype. We will also explore how nuclear actin influences the stabilisation of cell phenotypes through re-wiring DNA methylation, specifically 5'hmc by Tet2, to influence differentiation state and clinical outcome in pancreatic cancer.

Research Objectives
Defining the molecular mechanisms that influence cell fate will allow us to target the epigenetic plasticity behind tumour heterogeneity, progression and therapeutic resistance.
(ii) EON is an expert in hippo pathway signalling and epigenetics in pancreatic cancer and YS is an expert in epigenetic control of cell-phenotype in cancer.

This project asks three questions;

- How does RASSF1A regulation of nuclear actin affect chromatin at specific loci associated with pluripotency or differentiation?

Outcome: an understanding of how nuclear actin guides the movement of specific genes into regions of repressive chromatin or active transcription.

- Does mechanical force impact ATR-RASSF1A signalling to influence plasticity?

Outcome: defining specific extracellular or cytoplasmic cues that can trigger gene positioning and influence cell phenotype.

- Can targeting phenotypic plasticity improve therapy in PDAC?

Outcome: Can we promote differentiation in PDAC to improve therapeutic responses and survival?

Translational Potential
The potential of precision cancer medicine is limited by therapeutic resistance arising from tumour heterogeneity. Heterogeneity underpins cancer progression and results from a combination of genomic instability and epigenetic plasticity; the dynamic alterations of the epigenome responsible for establishing cell phenotype. The tumour microenvironment governs epigenetic plasticity but exactly how multiple states are generated and maintained unknown[7]. Personalised therapies targeting driver mutations are largely circumvented by the presence of genetically diverse resistant subclones. In contrast, epigenetic plasticity is reversible and an attractive target to prevent resistant phenotypes appearing or to revert phenotypes of recalcitrant populations (e.g. cancer stem-like cells) to improve overall therapeutic efficacy. Moreover, as plasticity in tumours can result in genome instability[8], the underlying alterations may highlight specific vulnerabilities not apparent from genetics alone. To understand how plasticity

occurs in tumours, we need to understand how the mechanisms governing cell phenotype are influenced by epigenetics and microenvironmental cues.

The genome kinases ATM and ATR phosphorylate RASSF1A-Ser131 to influence chromatin, transcription, and DNA replication. We now know that this influences plasticity and have shown how a SNP in *RASSF1* (rs2073498) encodes a mutation, RASSF1A^{A133S}, that disrupts phosphorylation at Ser131[9], blocks the formation of nuclear actin (preliminary data) and hinders differentiation. RASSF1A^{A133S} is prevalent in Caucasian populations with a minor allele frequency (MAF) of ≤0.17 in European cohorts and associates with early onset tumorigenesis in multiple cancers. We generated *Rassf1*^{A133S} mice that accelerate pancreatic and colorectal tumour models, supporting the hypothesis that RASSF1A maintains differentiation and prevents phenotypic plasticity in human tumours. This model gives us the opportunity to direct model an emerging pathological SNP in humans, while also provide a platform for strategies to intervene in hyperplastic phenotypic model.

Training opportunities

In addition to standard cell culture assays the candidate will receive training in high content and real-time microscopy, epigenetics (inc ChIPseq, bioinformatics), phase separation and transcription factories, nuclear F-actin filaments etc. In addition, there are opportunities to explore the in vivo relevance in mouse models of pancreatic cancer.

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2. Eyres, M. *et al.* TET2 Drives 5hmc Marking of GATA6 and Epigenetically Defines Pancreatic Ductal Adenocarcinoma Transcriptional Subtypes. *Gastroenterology* 161, 653-668 e616 (2021).
3. Chatzifrangkeskou, M. *et al.* RASSF1A is required for the maintenance of nuclear actin levels. *EMBO J* 38, e101168 (2019).
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7. Easwaran, H., Tsai, H.C. & Baylin, S.B. Cancer epigenetics: tumor heterogeneity, plasticity of stem-like states, and drug resistance. *Mol Cell* 54, 716-727 (2014).
8. Pefani, D.E. *et al.* RASSF1A-LATS1 signalling stabilizes replication forks by restricting CDK2-mediated phosphorylation of BRCA2. *Nat Cell Biol* 16, 962-971, 961-968 (2014).
9. Yee, K.S. *et al.* A RASSF1A polymorphism restricts p53/p73 activation and associates with poor survival and accelerated age of onset of soft tissue sarcoma. *Cancer Res* 72, 2206-2217 (2012).

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39. Modulation of tumour immunogenicity by IGF's in Prostate Cancer – [1] Dr Valentine Macaulay

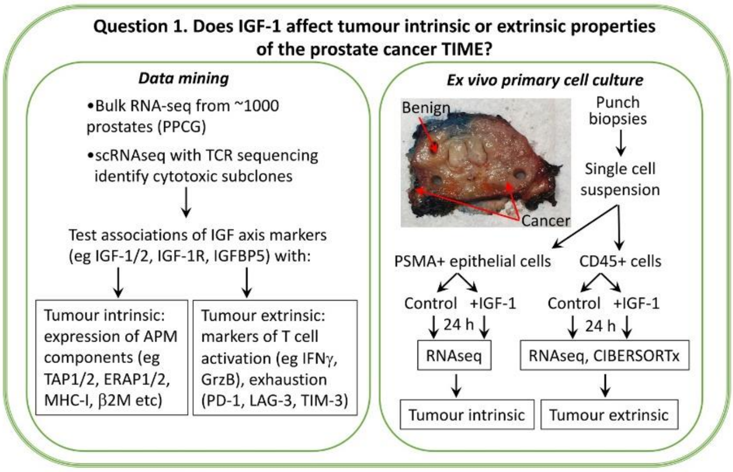
Primary Supervisor: Dr Valentine Macaulay
Additional Supervisors: Professor Tim Elliott
Eligibility: Track 1 students are eligible to apply for this project.

Abstract

Most prostate cancers have a 'cold' tumour immune microenvironment (TIME) with exclusion of tumour infiltrating lymphocytes (TILs) especially CD8+ cytotoxic T cells, defective antigen processing machinery (APM), and many immunosuppressive TILs eg regulatory T cells (Tregs). Cancer risk is affected by serum IGF-1 (sIGF-1): subjects with very low sIGF-1 show almost complete cancer protection, while high sIGF-1 increases prostate cancer risk. IGF-1 signals via IGF receptors (IGF-1Rs) to promote cell survival, invasion and androgen receptor (AR) activation. In other disease models IGF-1 enhances Treg function, while IGF blockade rescues APM components, inducing CD8 dependent anti-tumour immunity. We hypothesise that IGF-1 contributes to cancer risk via TIME immunosuppression. Using big data, patient derived explants (PDEs) and samples already obtained from men on a trial of IGF blockade pre-prostatectomy, we will ask whether IGF-1 affects: 1) tumour intrinsic or extrinsic TIME properties? 2) T cell phenotype/redistribution in prostate PDEs? 3) TIL localisation or exclusion? The findings may inform therapy and suggest novel approaches to prostate cancer risk reduction

Research objectives

- *Highly curated RNAseq data*: ~1000 primary prostate cancers (collaborator: Dr Woodcock, Pan Prostate Cancer Group, PPCG).
- *Prostate scRNAseq data* from the Macaulay lab and Dr Massie, Cambridge [1], with TCR sequencing to identify cytotoxic subclones and their expression of IGF axis genes.
- Patient derived explants (PDEs, ref 2) that maintain morphology, viability for ≤6 days (collaborators: Mr Lamb, Profs Verrill, Mills).
- WINGMEN samples including samples from 3 trial patients who were recruited to COMBAT Cancer (Dr Lamb, Prof Verrill, Dr Woodcock).



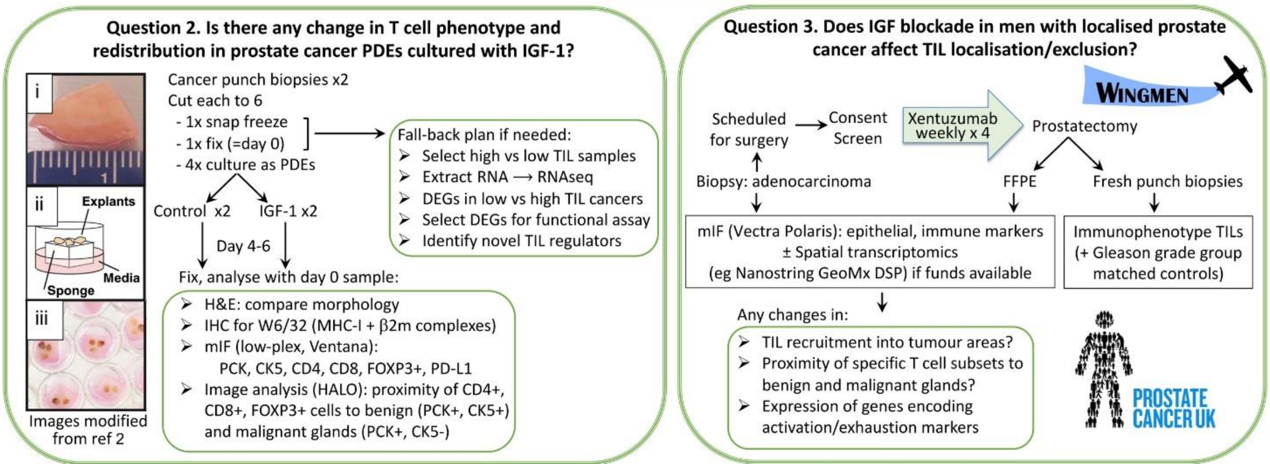
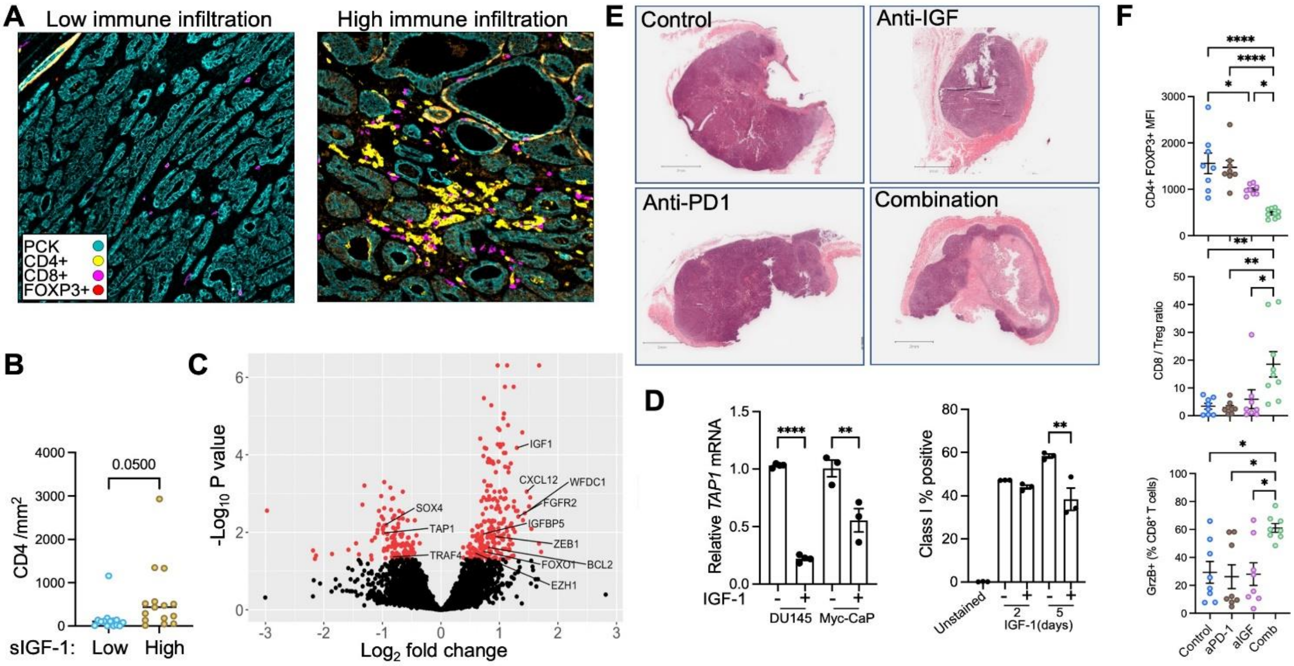


Figure 1. IGF-1 influences the TIME. A. Multiplex immunofluorescence (mIF) for pancytokeratin (PCK, epithelium), cytokeratin 5 (CK5, benign glands), immune markers. **B.** sIGF-1 associates with CD4+ TILs in prostatectomies of men with low (7.2-12.0 nmol/l) vs high sIGF-1 (21.7-31.5 nmol/l). **C.** RNAseq from same cases: differentially expressed genes (DEGs) in high eIGF-1 tumours include upregulated *IGFBP5* (indicates IGF-1R activation) and downregulated *TAP1* that transports antigenic peptides. **D**. IGF-1 downregulated *TAP1* mRNA in human DU145 and murine Myc-CaP cells (left), and reduced Myc-CaP cell surface Class I expression after 5 days (right). IGF-1 also upregulated PD-L1 in prostate cancer cells *in vitro* (not shown). **E-F**. Mice bearing Myc-CaP allografts treated with anti-PD-1, anti-IGF xentuzumab or combination. IGF:PD-1 co-inhibition increased tumour necrosis (E). FOXP3 TIL positivity was suppressed by anti-IGF-1 and PD-1:IGF co-inhibition (F, upper), and co-inhibition increased CD8+ cell/FOXP3+ Treg ratio and % granzyme B positive CD8+ TILs (F centre, lower).

The aims are informed by data (Fig 1) that the prostate cancer TIME is affected by sIGF-1 and by endogenous IGF-1 (eIGF-1) from epithelial and stromal cells. Supporting ***tumour extrinsic effects,*** sIGF-1 associates with CD4+ TILs (Fig1A-B), IGF blockade reduces FOXP3+ Treg TILs, and IGF:PD-1 co-inhibition increases CD8+/Treg ratio and GrzB+ CD8+ cells (Fig 1E-F). ***Tumour intrinsic effects*** are suggested by the correlation of high eIGF-1 with low TAP1 and Class I (Fig 1C-D) and by Prostate Adenocarcinoma Firehose Legacy data (n=500) where high eIGF-1 significantly associates with low TAP1 and TAPBP and high ERAP1 that trims peptides for presentation by MHC-I (not shown), potentially contributing to immune evasion by altering the peptide repertoire.



A plausible mechanism through which high IGF-1 promotes prostate cancer risk is therefore:
•High sIGF-1 and/or eIGF-1 upregulate PD-L1 and immunosuppressive cytokines in healthy or premalignant prostate tissue to promote Treg proliferation/function, resulting in a high tissue Treg setpoint.
•Tregs condition tissue in favour of immune suppression/exclusion, favouring progression to invasive cancer.
•CD8+ T cells are primed by tumour antigens eg those from AR ligand-binding domain (LBD). But in tissues conditioned to be immunosuppressive, primed TILs are suppressed/excluded, less able to control new cancers.
•IGF-1 deregulates APM components in tumour cells, further contributing to immune escape.
This mechanism suggests a tractable hypothesis to explain how the TIME is shaped by IGF-1:
•Tumour extrinsic effects that maintain Treg function and infiltration, suppressing CD8+ TILs
•Tumour intrinsic effects by regulating the APM, influencing ability of CD8+ TILs to recognise AR derived epitopes
•Thus IGF-1 prevents CD8+ T cell infiltration, localisation to malignant glands and lysis of prostate cancer cells.

The project will establish a new collaboration between Professor Elliott and Dr Macaulay, who will co-supervise the Clinical Fellow to investigate these hypotheses using the following models, samples and collaborations:

Depending on the findings, further investigations could include:

•Murine model *to assess TIL numbers, phenotype, intratumoral location.* 'HIT' mice (from Shoshana Yakar, NY) harbour a hepatic IGF-1 transgene with ~3-fold higher sIGF-1 [3]. Myc-CaP allografts in WT or HIT mice will be analysed by mIF, TIL phenotyping and RNAseq with digital cytometry (ImmuCC) to infer immune cell populations.

•Functional assays to investigate effects of IGF-1 or IGF blockade on i) T cell killing and suppression in co-cultures of prostate cancer cells and T cells, ii) AR antigenic priming and relative ability to prime in immunosuppressive environment, iii) immunopeptidomics to identify IGF-induced changes in antigen processing and presentation.

Translational potential: identification of a novel MOA for IGF-1 in the TIME has clear relevance to:

•***Therapy***: Few prostate cancers respond to immune checkpoint inhibition (ICI). If IGF-1 enables Tregs to suppress CD8+ TILs, IGF blockade may cause Treg suppressed CD8+ cells to be less exhausted and thus rescuable by ICI.

•***Cancer risk***: immunosuppressive actions of IGF-1 may contribute to the ability of high IGF-1 to enhance the risk of prostate cancer. Understanding this effect may suggest new approaches to risk reduction.

Training provided: The student will be trained in analysis of big data, explant and cell culture, flow cytometry and mIF. If resources become available, it may be possible to provide training in high-cost techniques to address our hypotheses at the single cell (scRNAseq, CyTOF) and tissue levels (CellDive, spatial transcriptomics).

References

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40. Understanding the mechanisms by which molecular and phenotypic heterogeneity of MLL-rearranged infant ALL affects clinical outcome–[1] - Assoc Prof. Anindita Roy

Primary Supervisor: Assoc Prof. Anindita Roy
Additional Supervisors: Philip Ancliff and Jack Batram
Eligibility: Track 1 students are eligible to apply for this project

Abstract
Although there has been much progress in treating acute lymphoblastic leukaemia (ALL) in children, there is still a subset of ALLs that have a very poor prognosis. This is particularly true for infants (i.e., children <1 year of age). Infant ALL (iALL) is frequently driven by translocations of the *Mixed Lineage Leukaemia* (*MLL* aka *KMT2A*) gene, which occur in ~80% of the cases, and represents a very aggressive type of leukaemia that is characterised by chemotherapy resistance and high relapse rates leading to a very poor prognosis[1]. The major challenge is that while iALL is aggressive, necessitating high-intensity treatment strategies, infants are extremely vulnerable to toxicity from chemotherapy and stem cell transplantation. It is clear that new approaches, underpinned by rigorous science, are required to improve characterisation, treatment stratification and outcomes. Recent studies have shown the benefit of upfront CD19 directed therapy in iALL[2, 3]. As there is no ongoing UK clinical trial for iALL treatment, the UK NCRI Leukaemia sub-group have designed an innovative treatment guideline using Blinatumomab to replace some of the most intensive and toxic chemotherapy blocks used in past protocols, as well as novel treatment stratification to assign post-blinatumomab therapy, carefully tailored to the biological features of each patient's leukaemia. These new guidelines present a unique and important opportunity to capture comprehensive clinical outcome data. Launching these guidelines as a national clinical study, accompanied by cutting edge scientific efforts to understand the molecular mechanisms underpinning the aggressive nature of iALL, will fulfil an unmet need in infant leukaemia care. We have formed a consortium of expert clinicians, scientists and parent advocates.

Research objectives
Aim 1: Delivering a national clinical study that will provide unique insights into optimal treatment pathways for iALL, to help inform the scientific and clinical agenda. **(P Ancliff/J Bartram)**

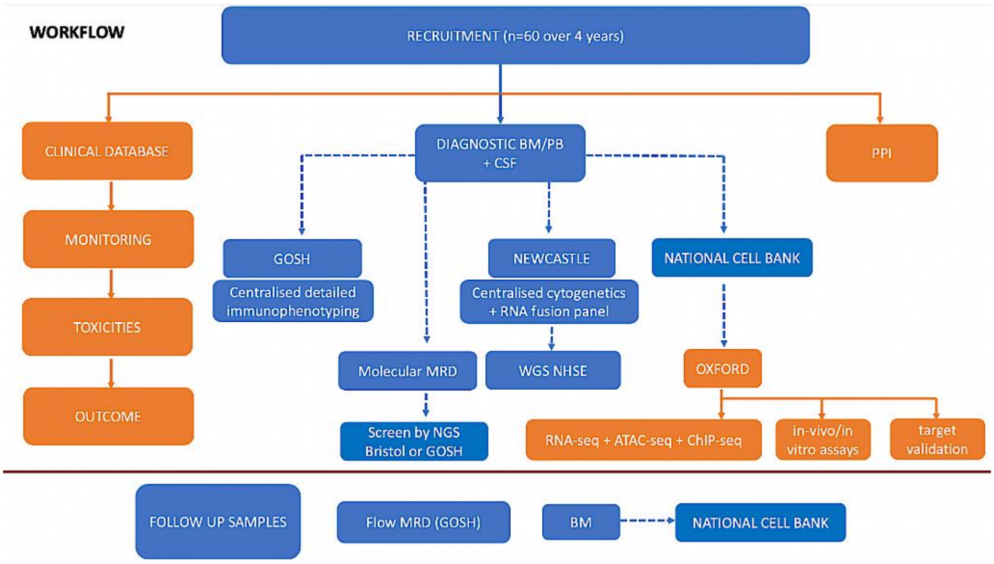

Figure 1: Schematic of project workflow. Sample collection and experimental workflow for the study. Blue boxes and arrows represent protocols that are covered by existing diagnostic pathways. Orange boxes/arrows represent protocols that require funding as part of this application. (BM: Bone marrow, PB: peripheral blood, MRD: minimal residual disease)

The national study opened in 2021. All newly diagnosed iALL patients are eligible for recruitment. There are c.12-15 cases of iALL in the UK/year. We expect to recruit >90% since all cases are presented at our National Leukaemia MDT. Expected recruitment is 60 patients over 4 years or longer, with the total recruitment period being 48 months. A secure central database held at GOSH will capture demographics, routine diagnostics (e.g. immunophenotyping, MRD, genetics), treatment, toxicity, and clinical outcome data. All patients will be assigned a UPN and patient identifiable data will not be made available to researchers, who will only receive linked anonymised samples. As part of this study, all diagnostic tests (as NHS standard of care) will be streamlined and undertaken in centres of excellence in a standardised manner (Fig 1).

Aim 2: Detailed characterisations of each patient's leukaemia, including biomarkers to better understand disease biology and response to treatment. **(collaboration T Milne)**

2.1 At diagnosis, all patient BM samples will undergo comprehensive characterisation as part of the diagnostic workup (Figure 1), including: i) detailed immunophenotyping to define the leukaemia-associated immunophenotype (LAIP), including the expression of myeloid antigens (S Inglott, Oxford and GOSH). This is crucial for follow-up and monitoring, especially detection of MRD and emergence of CD19-ve ALL relapse post-immunotherapy; ii) State of the art cytogenetics and RNA-fusion panel for detailed molecular characterization (Newcastle); iii) Ig/TCR rearrangement status by next generation sequencing to monitor MRD (GOSH). Whole genome sequencing (WGS) will be performed as a standard of care clinical test via the NHS Genomics Medicine Service/Genomics England, with access to raw data via the National Genomics Research Library.

2.2 To further unravel and understand the mechanisms by which molecular heterogeneity of MLL-rearranged infant ALL affects clinical outcome, we will perform comprehensive characterisation of diagnostic and relapse samples using multi-omic profiling. Samples will undergo RNA-sequencing, ATAC-sequencing and ChIP-sequencing for MLL-rearranged protein complex members, and key histone modifications such as H3K4me3, H3K27ac and H3K27me3 will be used to further define the global transcriptomic and epigenetic profile of the cells (A Roy/T Milne, Oxford). All work will be undertaken at the Roy/Milne labs where there is extensive experience of these techniques, including optimised protocols for small cell numbers.

2.3 Once specific molecular profiles/leukaemic subpopulations have been identified, including minor myeloid subclones that might cause plasticity or relapse; these will be correlated with a variety of clinical parameters and outcome to ascertain whether they are relevant prognostic biomarkers. To address the challenges of biomarker/target discovery in such a rare disease we will leverage established collaborations with the Interfant-21 trial (joint scientific committee has been set up, and additional funding secured through Fight Kids Cancer grant), and the HARMONY alliance (https://www.harmony-alliance.eu/) to validate identified biomarkers.

Aim 3: Identification of key vulnerabilities that may be targeted for novel therapies. **(collaboration T Milne)**

We will create and utilise comprehensively characterised primary patient material, in combination with state-of-the-art preclinical models[4], to provide unique insights into novel therapeutic and prognostic targets in iALL.

3.1 Full molecular characterization of iALL will give us the opportunity to study these cases in unprecedented detail at a multi-omics level. This will allow us to generate gene regulatory networks to identify key molecular pathways perturbed in iALL[5, 6] and how this correlates with clinical outcome. We will combine these findings with results of ongoing funded projects using CRISPR essentiality screens and molecular and functional screens in mouse models[4] (A Roy, T Milne Oxford).

3.2 We will validate targets by manipulating the expression of genes /pathways identified in 3.1, using in vitro and in vivo assays of primary iALL patient samples and an iALL model[4] (A Roy, Oxford).

Translational Potential

Since the last international trial for treatment of infant ALL (Interfant-06) closed in July 2016, infants diagnosed with ALL in the UK have not been enrolled in any clinical trials. While we will continue to work closely with our European and international colleagues, the UK is unable to participate in the next international trial (Interfant-21) as it has no randomisation arm. This leaves a vulnerable patient population without the benefit of a structured clinical trial in one of the poorest risk leukaemias. The current national study (2021 onwards) will ensure the implementation of risk-adapted treatment guidelines for all infants with ALL in the UK with the most comprehensive characterisation of the leukaemia and follow-up to date. We anticipate that patients will benefit immediately by the introduction of novel therapy approaches in our UK-NCRI-LSG guidelines, but it is imperative to capture real-life clinical data to learn about the efficacy and toxicity of these newer therapies compared to historic treatments. The study will allow us to monitor these treatment responses as well as investigate the biological mechanisms, which underpin its success or failure, including finding predictive biomarkers. This will lead to better stratification and a personalised medicine approach, which is a longer-term goal of this study. In addition, the phenotypic and molecular dataset generated will be an invaluable resource to clinicians and scientists treating patients or studying the biology of iALL. The most important feature of this project that will benefit patients is the true integration of clinical and scientific strategies in order to accelerate the development of effective therapeutic options.

Training Opportunities

The student will be embedded in the excellent scientific environment of the WIMM. He/she will be supported by experienced postdoctoral researchers in the lab, as well as collaborating labs (e.g. Milne) to learn cutting edge functional/molecular techniques such as flow cytometry, in vitro and in vivo assays, RNA-sequencing, ATAC sequencing, ChIP-sequencing, and bioinformatics. All of these techniques are already in use in our labs; and additional training opportunities will be available for computational analysis (via dedicated courses at the Centre for Computational Biology, WIMM). The student will be encouraged to take up the excellent training opportunities provided within the WIMM and the University of Oxford, to develop their research career. In addition they will gain experience in running a national clinical study including collating all clinical outcome and toxicity data under the guidance of expert clinical trial leads at GOSH.

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41. Describing T Cell recognition of tumours by machine-learning and statistical models [4] – Assoc Prof. Hashem Koohy

Primary Supervisor: Assoc Prof. Hashem Koohy
Additional Supervisors: Prof. Alison Simmons
Eligibility: Track 4 students are eligible to apply for this project.

Abstract
T cell recognition of a cognate peptide-MHC (pMHC) complex presented by infected/malignant and/or specialized antigen presenting cells is crucial for orchestrating a robust and sustained immune response. The molecular interactions between heterodimeric T Cell Receptors TCRs and pMHC ligands dictate the nature of the subsequent adaptive immune response. However, the underlying rules governing this engagement remain incompletely understood (1). A better understanding of TCR:pMHC interactions would allow further harnessing of adaptive T cell immunity, to assist in the development of vaccines and therapeutics and/or preventive strategies. This research in Koohy's group is focused on development of machine-learning and statistical models to help a better understanding the two key components of the interaction, namely the architecture and composition of the immune repertoire during the course of disease or treatment, and b) identifying the immunogenic T cell epitopes that can be used as targets for vaccines or immunomodulatory treatments. In this project, we will be investigating the extent to which T cells are involved in driving intestinal inflammation after Immune Checkpoint Blockade (ICB), and more importantly, the source of their antigen specificity. Additionally, we will be further exploring the statistical characteristics of T cell targets in cancers to derive accurate in silico predictions of cancer neoantigens (2).

Research objectives
The development of Immune Checkpoint Blockade ICB treatment for cancer, has revolutionized patient care. However, not all patients respond uniformly, and a subset experience immune-related adverse events irAEs, such as Checkpoint Induced Colitis CIC. Gastrointestinal inflammation, including CIC, affects a substantial proportion of patients treated with ICB, leading to treatment discontinuation. The underlying mechanisms of CIC remain poorly understood, although emerging evidence suggests dysregulated activation of T cells, potentially triggered by self-antigens or commensal microbes. Our ongoing collaborative project with Prof Alison Simmons and Dr Agne Antanaviciute has revealed clonally expanded CD8 T cells trafficked from blood to inflamed tissue in CIC patients. However, the antigen specificity of these cells remains elusive. Therefore, this proposal aims to identify dysregulated T cells and uncover the main source of their antigen specificity in CIC patients.

In a broader context, this project also focuses on modelling and identifying biomarkers of response to ICB. Numerous factors influence the immune response to treatment, including the mutation burden, cytotoxic T cell infiltration, and defects in antigen processing and presentation among others. Recent advances in high-throughput sequencing technologies particularly single cell techniques, enable the measurement of these features at the single cell level in patient samples at various time points, spanning pre-treatment, treatment and post-treatment. To study the mechanisms underlying response heterogeneity, we employ statistical and machine learning models trained on high thought put sequencing data encompassing genomics, transcriptomics, T cell receptor repertoire and epigenomic data. However, due to limited data availability, many potentially predive features have not yet been integrated into statistical and computational approaches. Consequently, the scientific community requires more sophisticated methods capable of modelling confounding factors and leveraging comprehensive patient data.

Within our research group, we capitalize on the expertise of in-house specialists to generate diverse types of data and develop next generation mathematical and computational models for exploring the role of aforementioned factors in response heterogeneity. Additionally, we aim deorphanizing T cell receptor from cancer patients and gain insights into the underlying rules governing T cell interaction with tumours.

Translational Potential
Although cancer immunotherapies have revolutionized cancer treatment, not all patients respond equally and about 60% patients develop gastrointestinal inflammation. This project may inform the development of novel biomarkers predictive of inflammation and more broadly inform the development of treatment approaches requiring tailoring of peptide TCR interactions.

Training Opportunities

The project offers training in T cell immunology, single cell technologies, their applications in immunology as well as their specific computational challenges. Successful delivery of the project will also require a good understanding of machine-learning and statistical inference preferably Bayesian Statistics. Training for both immunology and modelling will be provided by ourselves in the unit or within the Oxford University teaching and training schemes. We closely work with our collaborators including: Prof Alison Simmons[3,4,5] and Dr Agne Antanaviciute to leverage the data from high-throughput technologies to investigate the molecular mechanisms of checkpoint colitis (3) and to study the heterogeneity and composition of intestinal antigen experienced immune cells mirrored by the composition of intestinal microbiome and their relevance to the outcome of the immune checkpoint blocked treatment. This is therefore a great opportunity to learn more about the applications of cutting-edge experimental single cell and spatial transcriptomics in action in addressing immunological and medical problems.

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42. Urological cancers beyond the microscope; novel multiomic analysis of features associated with DNA instability and the tumour immune micro-environment [1,2,3,4] – Assoc Prof. Clare Verrill

Primary Supervisor: Assoc Prof. Clare Verrill
Additional Supervisors: Prof. Ian Mills
Eligibility: All tracks are eligible to apply for this project.

Abstract
Pathology is the study of disease and histopathologists analyse tissue samples to make diagnoses and assessments of how aggressively cancers might behave. Despite extensive specialist expertise and international efforts to optimise these assessments, they are inherently limited by the performance of human based observers. Current human based grading systems are imperfectly predictive and prognostic of disease behaviour. In addition, despite scientific advances stressing the importance of the tumour immune microenvironment, this feature is not routinely assessed as part of the diagnostic process, leaving large amounts of valuable information untapped.

Combinations of novel and powerful technologies such as spatial transcriptomic and AI image-guided whole genome sequencing have the potential to unlock new insights into disease biology. Some urological cancers lag behind many other cancer types in terms of understanding of disease behaviour, microenvironment and molecular drivers, consequently routine molecular testing is generally not currently undertaken as with other tumour types e.g. lung (ALK-1, EGFR).
Kidney and bladder cancers (renal cell carcinoma and urothelial carcinoma respectively) receive relatively little research investment and have poorer outcomes than many other cancers. These cancers are now eligible for immunotherapies such as PDL-1 inhibitors, but with variable success, which may in part be due to a lack of understanding of the tumour immune environment.

In this project we will undertake highly detailed multi-omic profiling (Nanostring GeoMx, AI image guided whole genome sequencing) of up to 20 kidney and bladder cancers. This discovery data set will be mined for the most scientifically valuable areas of the tumour and immune microenvironment and the most useful markers of particular populations of immune cells.
In spite of their utility in providing biological insights, detailed multi-omic profiling is not feasible at scale. Hence we need to translate these into pipelines that can be resourced routinely, such as immunohistochemistry or even from morphological changes detected by AI on readily available H&E sections. We aim to test the most promising image analysis and immunohistochemical targets in a larger cohort of up to 500 cancers.

Ultimately this will enable us to look at the relationship between genomically unstable cancer cells, other cancer cells and the immune environment.

Research objectives

Academic value
i) Discovery - Detailed genomic, transcriptomic and image analysis AI profiling of up to 20 kidney and bladder cancers (renal cell carcinoma and urothelial carcinoma respectively) with a particular emphasis on morphology and the immune microenvironment. Identify the scientifically most valuable areas within cancer cells and the immune cells themselves.
ii) Testing - Select from the discovery big data set, a panel of the scientifically most useful immunohistochemical immune or other tumour markers that indicate which particular populations are present and apply to up to 500 kidney and bladder cancers.
iii) Prediction and validation - Create AI based image analysis signatures (proxies) from H&E alone of the scientifically most valuable morphological changes and immune populations and test in a new validation cohort.
Cohorts with clinical outcomes, samples, patient consent and ethical approvals are all available and ready to use via previous initiatives.

Outcomes
i) Big data discovery set. Up to 20 kidney and bladder cancers with detailed -omic profiling using the Nanostring spatial transcriptomic platform, image guided low-pass whole genome sequencing (for copy number unstable cancer cells) and AI

based image analysis. This will guide the selection of a targeted panel of immunohistochemistry that will be undertaken on a larger scale (up to 500 cases) and analysed together with the routinely available morphology from H&E stains.
ii) From (i) we identify the scientifically most valuable regions of interest in the cancers and including in the immune microenvironment and evaluating the inter-play between cancer and immune cells. We then aim to translate this into H&E based morphological signatures derived by image analysis AI that could be translated into clinical use at scale.

Collaborators

In this project, the DPhil candidate will work with a unique team of urological histopathologists (Verrill), scientists (Mills, Rao), data scientists (Woodcock) and engineers with international expertise. The team have a number of established collaborative projects in this field and have created an exciting programme of work around histogenomic associations linking novel AI based image analysis with molecular based genomic and transcriptomic sequencing (bioRxi). Issa and Hester (TRIG) bring expertise in the tumour microenvironment. Mills is a highly experienced DPhil supervisor.

The programme leverages significant investment made via the AI Imaging Centre of Excellence ''PathLAKE'' with Oxford hosting one of the UK's first fully digital NHS histopathology laboratories with live clinical AI technologies [ref press release]. The project also builds on cohorts and collaborative work with industry partner Janssen Biotech Inc. (Cartography) and pump priming funds from the University of Oxford Medical and Life Sciences Translational Fund.

Translational potential

The project will create novel objective and quantitative ways of analysing urological cancers above and beyond that which can be achieved with human-based pathological assessments which are largely qualitative and inherently subjective. In addition, there will be particular emphasis on the immune microenvironment which is currently not analysed routinely in these cancers. Ultimately the findings will be evaluated by the end of the DPhil for the most promising which in the medium to longer term can be further developed and leverage further funding via market opportunities or funding bodies.

Training opportunities

The successful candidate will gain a detailed understanding of urological cancer histopathology (Verrill). They will be able to gain laboratory experience within histopathology (sectioning, immunohistochemical staining), whole genome sequencing and bioinformatic analysis (Rao), spatial transcriptomic experimental design and analysis (Issa, Hester), integration of diverse datasets and machine learning (Woodcock) and deep learning for image analysis.

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43. Investigating the role of the ubiquitin ligase BIRC6 in aneuploid glioblastoma cell survival [1,2,3] – Dr Paul Elliott

Primary Supervisor: Dr Paul Elliott
Additional Supervisors: Dr Vincenzo D'Angiolella
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Abstract

Glioblastoma is a tumour with a dismal prognosis and few therapeutic options. The tumour is characterised by profound heterogeneity, fuelled in part by the presence of rampant genomic instability. Chromosomal instability is very frequent in glioblastoma cells and, so far, targeting aneuploid cells has proven difficult. A recent study identified co-essentiality of the ubiquitin ligases UBA6 and BIRC6 in a subset of tumours with high aneuploidy through a role in the integrated stress response and cell death (1). However, detailed understanding of mechanisms underpinning this dependency on BIRC6 and UBA6 are not known.

BIRC6 is a large (~5000 amino acid) E2/E3 ubiquitin ligase of the Inhibitor of Apoptosis family. Our recent structural and biochemical work has uncovered BIRC6 directly restricts executioner caspase-3 and -7 and through working exclusively with the non-canonical E1 UBA6, ubiquitinates caspase-3,-7 and – 9 and other substrates (2). Thus, BIRC6 seems to be a crucial sensor of aneuploidy to balance cell death decisions in aneuploid cells. The DPhil project will investigate how BIRC6 supports aneuploidy cancer cell survival particularly focussing functional biological studies on glioblastoma (3). The project will uncover the BIRC6–substrate interactome in glioblastoma and will biochemically, structurally and functionally characterise these interactions providing key insights into how BIRC6 enables aneuploid glioblastoma cell survival, crucial for informing novel future glioblastoma therapeutic strategies.

Research objectives

Objective 1: Uncovering BIRC6 regulated proteome in glioblastoma cell lines

To gain a mechanistic understanding of how BIRC6 enables aneuploid glioblastoma cell survival, we will determine the proteome regulated by BIRC6 in four glioblastoma cell lines chosen for differing levels of BIRC6 dependency and aneuploidy level. Through cutting-edge proteomics combined with Tandem Mass Tag (TMT) labelling, we will quantitatively compare complete proteomes of these cell lines upon knock-down of BIRC6. Gene ontology analysis will be used to identify pathways in which differentially regulated proteins are involved and thus regulated by BIRC6 in glioblastoma. Proteins that display most significant upregulation in BIRC6-dependent glioblastoma will then be the focus for the downstream objectives in which we will establish how each substrate identified contributes to regulate survival of glioblastoma cells.

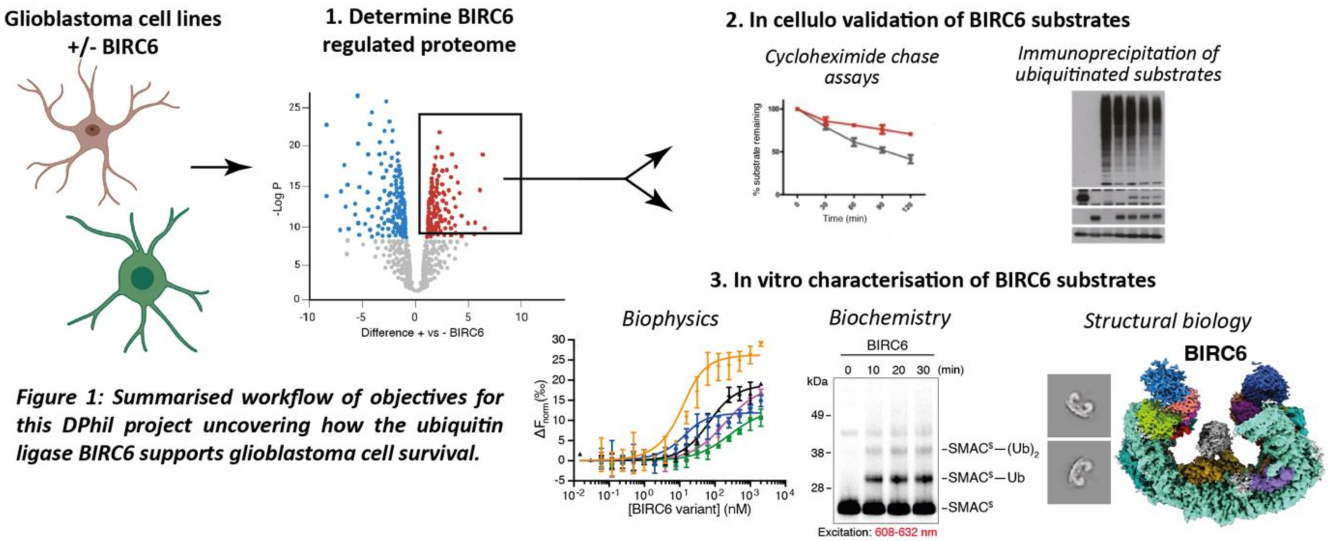

Figure 1: Summarised workflow of objectives for this DPhil project uncovering how the ubiquitin ligase BIRC6 supports glioblastoma cell survival.

Objective 2: Validation of BIRC6 substrates in glioblastoma cell lines

The most significantly upregulated proteins identified in Objective 1 will be assessed for capacity as BIRC6 substrates. This will be achieved through cycloheximide chase assays testing either endogenous substrates or epitope tagged substrates ectopically expressed at near endogenous levels to confirm substrate stability upon BIRC6 depletion. Candidate substrates identified will then be verified for interaction with BIRC6 in cellulo through immunoprecipitation methods. Further to these experiments, substrate degradation via ubiquitination will be confirmed through use of Tandem Ubiquitin Binding Entities (TUBEs) to immunoprecipitate ubiquitinated substrates followed by ubiquitin linkage type identification using established chain selective deubiquitinase profiling methods employed in the Elliott Lab. The substrates identified will also be analysed for their role in controlling DNA damage, chromosomal mitotic segregation and apoptosis. While we have chosen transformed cancer cell lines as initial models, we will confirm the findings in glioblastoma stem cell models with basal aneuploidy and/or induced aneuploidy, well established in the D'Angiolella Lab. Non-transformed neural stem cells will also be used to establish dependency of BIRC6 in normal tissues.

Objective 3: In vitro characterisation of BIRC6 substrates

Confirmed substrates will then be cloned, recombinantly expressed and purified through either *E.coli* or Sf9 expression systems. Recombinant substrates will be tested for ubiquitination by BIRC6 in established in vitro substrate ubiquitination assays using recombinant E1 ubiquitin ligase (UBA6) and fluorescently-labelled ubiquitin. The interactions between BIRC6 and novel substrates will then be characterised through structural biology methods including cryoEM and biophysical techniques well established in the Elliott Lab. Structure-function insights will be explored further through use of existing BIRC6 mutations where relevant or design of new structure-guided mutations.

Outcomes: This DPhil project will determine the BIRC6 substrate interactome in glioblastoma and will uncover how BIRC6 substrate specificity is achieved and how ubiquitination of these substrates enables glioblastoma cell survival. Together this project will provide the first detailed mechanistic insights into BIRC6 cellular function in aneuploid glioblastoma survival

Translational potential

This project will provide crucial detailed insights into how BIRC6 supports aneuploid glioblastoma cell survival. We have the longer-term vision of translating our cellular, structural and biochemical knowledge obtained to develop small molecule compounds that disrupt BIRC6 interaction with identified substrates for therapeutic use. Furthermore, considering most cancers feature aneuploidy, BIRC6 and its substrate interactome may be key molecular anti-cancer targets; therefore, through this project, the detailed cellular and molecular insights into understanding how BIRC6 is exploited in aneuploid glioblastoma will be important for informing cancer therapies more broadly.

Training opportunities

This DPhil project provides an exciting opportunity to combine cancer cell biology methods with mass spectrometry and biochemistry, biophysics and structural biology techniques. The student will join a dynamic team and will develop a broad range of skills including: proteomics analysis methods, protein purification techniques, in vitro biochemical ubiquitin-related assays, structural biology (cryoEM) and, through working closely with Dr D'Angiolella's Laboratory, glioblastoma cancer cell biology methods. The student will also have multiple opportunities to present their findings at inter-departmental seminar series and national and international conferences.

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44. Characterising the NK/Myeloid crosstalk during tumour immune escape [3] – Assoc Prof. Audrey Gérard

Primary Supervisor: Assoc Prof. Audrey Gérard
Second Supervisor: Claudia Monaco
Eligibility: Track 3 students are eligible to apply for this project.

Abstract

Cancer cells can be recognised and killed by our immune system. However, tumours developed strategies to evade the immune system by inhibiting the main immune cell type that can kill them, called CD8 T-cells. In recent years, immune therapeutics emerged to counteract this, called checkpoint blockade. This treatment aims to reinvigorate CD8 T-cells and has shown unprecedented success in treating aggressive cancers. However, some patients who initially responded to checkpoint blockade become refractory. One of the reasons is because cancer cells once again evade the attack induced by the CD8 T-cells reactivated by checkpoint blockade. Often, they do so by mutating and blocking the effect of a specific immune mediator, IFNγ. This project aims to understand whether the immune system can adapt to those mutated tumour clones, and whether we can leverage this to reinvigorate the immune system yet again. Our data show that immune cells other than CD8 T-cells become activated following this secondary escape. They are monocytes and NK cells, and importantly, they also have the potential to kill cancer cells. We will study the crosstalk between monocytes and NK cells to understand if it is beneficial or detrimental to anti-tumour immunity. To do this, we will recreate IFNγ-dependent tumour escape and explore when the NK/monocyte crosstalk arises, where it happens and the effect of manipulating this crosstalk on the anti-tumour response. The goal is to understand how to leverage this newly generated immune response to provide alternative strategies to control tumours.

Research objective

Tumours actively escape the immune system by inducing an immunosuppressive state where intra-tumoural CD8 T-cells are "exhausted", lacking effector functions. Checkpoint blockade is a therap designed to reinvigorate those exhausted T-cells. It has shown unprecedented success in treating aggressive cancers such as metastatic melanoma[1]. But the response rates are still only 15 to 30%, in part because tumours have developed escape strategies checkpoint blockade. The most reported pressure in this context is induced by CD8 T-cells. Patients who progressed after initially responding to checkpoint blockade often carry tumours deficient in IFNγ signalling pathway[2]. IFNγ exerts cytotoxic or cytostatic effects on tumours and induces Major histocompatibility complex (MHC) expression. MHC expression is necessary for CD8 T-cells to recognise and kill tumour cells. Therefore, down-regulation of the IFNγ pathway leads to tumour escape by inhibiting T-cell recognition. How the immune system responds to this has been largely overlooked. They however have the capacity to do so. For example, we recently found that CD8 T-cells sense

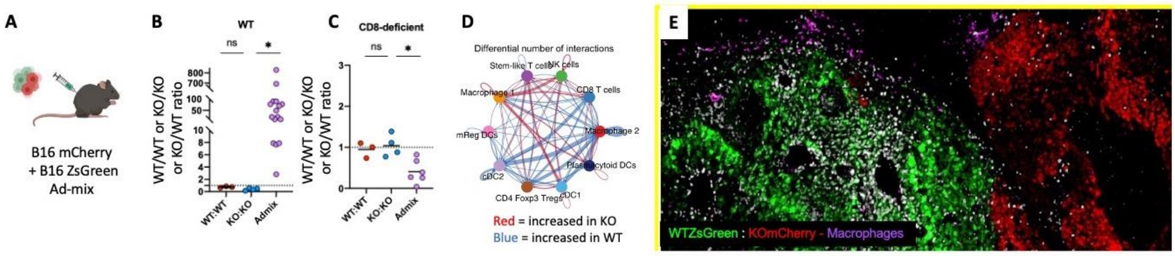

Figure 1: Immune adaptation to IFNγ-dependent tumour escape. A- Control (WT-Zsgreen) and IFNγRKO (KO-mCherry) B16 melanoma tumour cells, were ad-mixed, as indicated, and engrafted in WT mice. (B-C) After 12 days, tumours were harvested, and single cell suspension obtained. Graph shows the ratio between the different tumour types, quantified by flow cytometry in WT (B) and CD8-deficient (C) mice. Every dot is a mouse. D- We performed single cell RNA sequencing on immune cells from WT and KO tumours. The graph displays the putative strength of interactions between immune cell subsets. Cells linked by blue lines represent communication observed in WT tumours. Cells linked by red lines represent communications observed in KO tumours. E- Ad-mixed WT and KO tumours were fixed and imaged by confocal microscopy. Macrophages (purple) were stained with F4/80 antibodies.

IFNγ present in the tumour microenvironment, which restricts their anti-tumour response[3]. The paradigm is that immune cells, in particular T-cells, are inhibited or simply can no longer recognise cancer cells. To what extent immune cells adapt to tumour escape and whether this can be leveraged, is unknown. In this context, NK cells have emerged as a potential immune target to trigger an immune response against tumour cells that downregulated the expression of MHC molecules. But we don't know how cancer cells and the immune microenvironment affect NK cell fitness.

To recapitulate escape, we use a simple system where we engraft mice with an ad-mix WT tumour clones and "escapee" tumour clones that do not express the IFNγR (KO). Each clone expresses a different fluorescent protein (ZsGreen or mCherry) to track them (Fig.1A). In this model, KO tumour clones indeed escape and overtake WT clones (Fig.1B). This escape does not happen in mice that do not produce CD8 T-cells (Fig.1C), as CD8 T-cells kill WT tumours rather than KO tumours. However, NK cells, which have been suggested to take over and kill KO tumours, do not seem to do so efficiently. Sequencing of the immune microenvironment of WT and KO tumours demonstrates that escape is characterised by the emergence of crosstalk between NK and Macrophages (red lines, Fig.1D). Most macrophages in tumours are inhibitory, and we previously visualised their pro-tumour function during metastasis[4]. The overall hypothesis is that the NK/macrophage crosstalk inhibits NK cell fitness, and that we can find strategies to target this crosstalk to improve anti-tumour immunity against escape mutants.

Research Objectives:

i) Explore the emergence of the NK/Macrophage interplay as escape occurs and its potential implication on escape efficiency. You will harvest tumours at different time points and correlate the phenotype of NK and myeloid cells with the "extent" of tumour escape. Tumour escape can be quantified by tumour growth and the ratio between WT and KO tumours either by flow cytometry or using IVIS spectrum in vivo imaging. Cell phenotype can be assessed by high-dimensional flow cytometry. You will also deplete NK cells or myeloid cells and study how they influence tumour escape.
ii) Investigate the co-dependency and mediators of the NK/Myeloid crosstalk. Using ex vivo functional assays, you will study NK cell fitness over time, as escape occurs, and the relevance of myeloid cells for NK functions. Using our already generated transcriptomics dataset, you will identify and test candidate pathways that can interfere with NK differentiation and function. For example, you will test known mediators of NK/Myeloid cell communication such as IL12/18/15, based on our preliminary analysis of transcriptomics data pointing to IL18 as a mediator of NK/Myeloid cell communication in IFNγRKO tumours.
iii) Explore the spatial relationship between tumour escapees and the immune response to understand how it fuels tumour escape. Newly generated data in the lab demonstrate that IFNγ-dependent escape leads to segregation between WT and KO cancer cells. We hypothesise that KO and WT regions are populated by distinct immune states, potentially contributing to KO tumour escape. Preliminary analysis shows that Macrophages are located at the margin of the tumour (Fig.1D). In addition, our scRNAseq data suggest that NK cells and Macrophages attract each other and reside in the same niche, excluding NK cells from the core of the tumours. We will define the localisation of NK cells and different myeloid populations in WT, KO, or admixed tumours. To analyse immune cells of interest in different regions, you will use confocal microscopy and light-sheet microscopy, available at the Kennedy Institute. We hypothesise that NK cells are colocalised with Macrophages at the periphery of tumours, restricting their action.

Outcome: This project will unravel the relevance of the NK/Macrophage crosstalk for tumour escape, identifying key pathways by which immune cell sense and adapt to changes in the tumour landscape. This will uncover potentially targetable new mechanisms by which tumours respond to immune pressure.

Translational potential

This project will help us understand the relevance of the NK/myeloid interplay on tumour escape and whether we can leverage our acquired knowledge on this pathway to counteract IFNγ-dependent tumour escape. The recent discovery that down-regulation of the IFNγ pathway is a major escape mechanism secondary to strong immune pressure makes this project timely and relevant. Understanding how IFNγ reshapes anti-tumour responses will be key to offer new strategies to overcome immune escape. NK cells are often found in small numbers in tumours, and therefore have not received much attention. But they emerge as key to control MHClow tumours, and we need to better understand how to increase their homing to tumours and which signals lead to their inhibition. This is important, and multiple cellular therapies aim to use NK cells.

Training opportunities

The student will be based at the Kennedy Institute of Rheumatology which is a world-renowned institute and is housed in a state-of-the-art research facility. This project provides broad training in cancer biology and immunology covering a range of cellular, molecular and functional immune assays. Students have access to cutting-edge technologies such as disease mouse models of cancer, NK/Monocytes co-cultures systems, multiplex imaging.

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45. The impact of Hypoxia on HLA-E surface expression and peptide presentation[3] – Assoc Prof. Geraldine Gillespie

Primary Supervisor: Assoc Prof. Geraldine Gillespie
Second Supervisor: Prof. Jane McKeating
Eligibility: Track 3 students are eligible to apply for this project.

Abstract
Human leukocyte antigen E (HLA-E) is a non-classical MHC protein whose primary function involves the regulation immunity via an NK cell axis [1]. This system involves HLA-E binding to conserved 9 amino acid leader sequence peptides (termed 'VL9') from classical major histocompatibility complex class I (MHC Ia) proteins [2]. Once expressed at the cell surface, HLA-E-VL9 complexes are recognised by inhibitory CD94-NKG2A receptors expressed on NK cell. When MHC Ia expression is absent or reduced, during viral infections or following malignant transformation to evade T cell recognition, limited VL9 leader peptide availability reduces HLA-E-VL9 surface expression, causing loss of NKG2A binding, and subsequent NK cell-mediated attack. However, there are contexts where HLA-E can present unusually diverse, non-VL9 peptides to CD8+ T cells, suggesting an unanticipated role for HLA-E in the context of T cell immunity [3,4]. This previously undiscovered role is exciting and offers potential for 'universal' vaccine development as only two functional allotypes of HLA-E in humans have been reported in contrast to the enormous diversity of MHC Ia allotypes. Should HLA-E share the same ability to target broad, sequence diverse peptide repertoires, it offers an alternative and exciting platform for novel therapeutic strategies, especially for tumours where HLA-E is up-regulated. One of the many important factors affecting the physiology of cancer cells is the local oxygen tension. Hypoxia or low oxygen levels are a hallmark of solid tumours and associate with poor prognosis. A hypoxic microenvironment enhances the proliferation and invasiveness of tumour cells, impairs drug delivery and promotes evasion of host immune responses. Hypoxia can negatively regulated MHC Ia expression, however, the impact on HLA-E has not been explored.

Research objective
The tumor microenvironment comprises the tissue surrounding and interacting with the tumor such as extracellular matrix, vasculature, stromal cells, and immune cells, as well as the oxygen concentration and pH levels. Hypoxia develops as a proliferating tumor outgrows its surrounding vasculature leading to reduced oxygen levels. Hypoxia-inducible factors (HIFs) regulate oxygen homeostasis and activate the transcription of many genes involved in angiogenesis, metastasis, and immune suppression. To be recognized by CD8+ T cells, antigens need to be processed by the antigen processing and presentation machinery and presented on the cell surface in complex with a MHC Ia molecule. Hypoxia has been reported to regulate MHC Ia in a cancer type-specific manner with some studies reporting an upregulation in melanoma and colorectal cancer cell lines [5,6]. In contrast other studies report a hypoxic mediated down-regulation of MHC-I in sarcoma and pulmonary tumor mouse models [7], non-small-cell lung cancer models [8] and multiple myeloma [9]. A single report reported that hypoxia increased HLA-E [10], highlighting a need for further studies. **We hypothesize that a hypoxic tumour microenvironment will affect the cell surface expression and/or alter the peptide presentation profiles of HLA-E on cancer cells.**

This project will examine the effect of hypoxia on HLA-E expression in various cancer cell lines such as human papilloma virus associated cervical cancer cell lines, hepatitis B virus infected human hepatoma cells with viral integrants. In collaboration (Prof. N. Ternette) we will explore the impact of hypoxia on the HLA-E peptide repertoire using mass spectrometry. Parallel experiments will study the efficacy of pre-defined HLA-E restricted T cells recognition of tumour cell lines (for which we have previously defined cancer epitopes) under hypoxic conditions. We have access to a range of pharmacological agents and licensed drugs that modify HIF activity to define underlying mechanisms. An understanding of the relationship between HLA-E and hypoxia, and the molecular biology underlying altered expression would provide a significant advance in our understanding of a topic that is currently neglected, but crucially important from a cancer-targeting perspective.

Translational potential
The near monomorphic nature of HLA-E combined with its dysregulation on certain cancer cells highlights its potential as an immunotherapeutic target. Understanding the impact of hypoxia on HLA-E and the peptide repertoire presented could facilitate targeting by peptide specific T cells or by antibodies that recognise peptide-HLA-E (TCR 'mimics'). If instead, HLA-E is downregulated, this information could advance targeting of cancer cells via NK cell-based approaches. Either of these approaches could be combined with pharmacological agents that modify HIF activity to amplify the relevant immune-

therapeutic targeting. Elucidating the effect of hypoxia on HLA-E surface expression and antigen presentation provides new therapeutic opportunities to harness and regulate different arms of the immune response.

Training opportunities

This project will be co-supervised by Geraldine Gillespie and Jane McKeating who provide complementary expertise in HLA-E biochemistry, structural biology, T cell immunology, molecular virology and hypoxia biology. The interdisciplinary project will provide a unique training environment in cancer immunology and range of techniques will be offered including molecular, biochemical and in vitro models of T cell suppression of cancer cells. Transferable skills including oral presentations at joint lab meetings, critical reviewing of published scientific literature by contributing to journal clubs and scientific writing by reviewing and drafting manuscripts for publication.

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46. An interdisciplinary approach to understand how interactions between proliferating and invasive melanoma cells can promote metastasis [4] – Prof. Ruth Baker

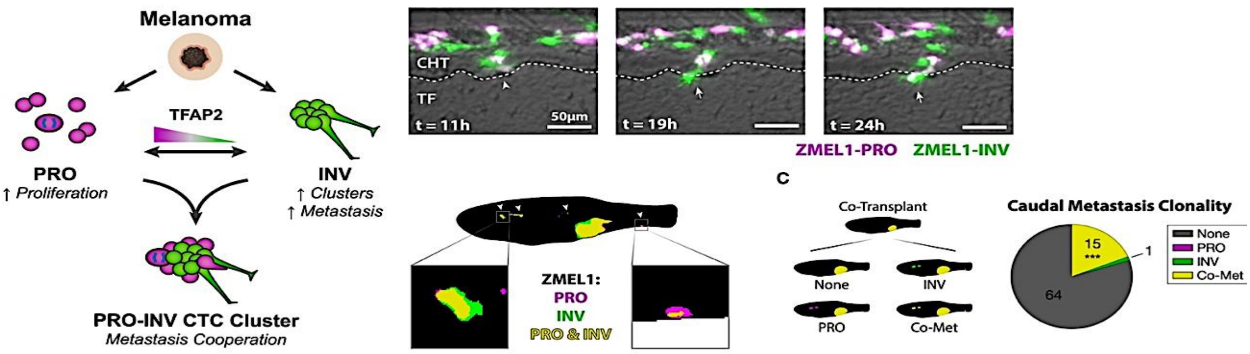
Primary Supervisor: Prof. Ruth Baker
Second Supervisor: Prof. Helen Byrne and Prof. Richard White
Eligibility: Track 4 students are eligible to apply for this project.

Abstract

Analysis of patient biopsies shows that melanomas are highly heterogeneous at both genetic and epigenetic levels. Single-cell sequencing has demonstrated at least five co-existing transcriptional cell states, including distinct proliferative versus invasive states. However, little is known about how these states coexist within tumours, whether they interact to promote metastasis and tumour progression, and/or whether cells switch between these states. The overarching aim of this project is to combine mathematical models with experimental data collected in the White Lab to provide new insights into these challenging questions.

Research objective

Background: Melanomas are heterogeneous, with multiple cellular phenotypes characterised by distinct transcriptional states [2,4]. At the extreme ends of these states are proliferative versus invasive subpopulations usually linked to the "go or grow" hypothesis. Recent *in vitro* experiments performed in the White Lab have shown that during metastasis, melanoma cells from these proliferative and invasive subpopulations spontaneously form spatially structured clusters, with invasive cells surrounded by an outer rim of proliferating cells. Additional *in vivo* experiments show that heterogeneous clusters, comprising proliferative and invasive cells, form secondary tumours, or metastases, at rates which are significantly higher than those associated with each subpopulation.



The mechanisms giving rise to these seemingly counter-intuitive observations are not well understood. Yet a better understanding of the process of metastasis will be key to the development of novel cancer therapeutics.

Aims and objectives: As such, the aim of the project is to develop mathematical models to analyse, interpret and integrate dynamic imaging data at the tissue scale, and high-resolution spatial transcriptomic data, from both *in vitro* and *in vivo* experiments. Repeated rounds of model-driven hypothesis generation and experimental validation will provide new understanding of how melanoma cell clusters form, and how their ability to metastasise depends on both the extent of heterogeneity and the spatial structure of the clusters. The project will initially consider just two cellular phenotypes, proliferative and invasive [2]. Subsequent extension of the models to include the five co-existing transcriptional cell states detailed in [4] will enable comparison of how the extent of phenotypic heterogeneity impacts metastasis. The objectives are:

1. To develop models of cluster formation based on the coagulation-fragmentation framework [5], and to calibrate models to quantitative data collected in the White Lab using computational Bayesian statistics approaches, to understand the mechanisms driving observed cluster size distributions.
2. To develop spatially resolved, cell-based models of cluster formation [1,3], in which individual cellular properties (such as rate of cell cycle progression and invasiveness) can be specified and, through careful calibration of the model to experimental data, to explore the mechanisms that give rise to the observed spatial structure of clusters.

3. To use the models developed in Objective 2 to predict how metastatic potential changes with cluster composition, and the extent of cooperation between the different cellular subpopulations.

Academic value of the research and funding justification: Funding for a DPhil student will enable us to transform understanding of how melanoma cell heterogeneity impacts metastatic potential. Progress on this challenging problem requires a multidisciplinary team with expertise across mathematical modelling, computational statistics, experimental biology, and image analysis. The funds will cover the costs of a graduate student who will carry out research in the Mathematical Institute.

Collaborations: The project will initiate a new collaboration between Professors Baker, Byrne and White. The student will make frequent visits to the White Lab where they will interact with lab members investigating melanoma metastasis. This will enable the student to learn the relevant melanoma biology, experimental techniques, and data analysis methods, and to contribute to experimental design. The team will meet with the student on a weekly basis.

Translational potential

This project will provide new insights into the processes driving melanoma metastasis, through the interrogation of large-scale data sets using mathematical modelling and computational statistics. It will generate new methodologies for analysing multi-model data and strengthen expertise in multidisciplinary approaches to tackling cancer. As such, it will contribute to the scientific themes “Cancer big data” and “Early cancer detection”.

Training opportunities

The student will receive training in mathematical modelling using differential equations, stochastic processes and cell-based models, as well as computational Bayesian statistics, image analysis, experimental design, and multidisciplinary research. Within the Mathematical Institute, the student will be part of the Wolfson Centre for Mathematical Biology, where they will take part in weekly mathematical oncology focus meetings and research skills training sessions, and attend formal weekly seminars. The student will also spend time in the White Lab, attending group meetings and relevant seminars at the Ludwig Institute for Cancer Research. Here, they will also have the opportunity to learn how to perform *in vitro* cell-based assays, if desired.

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47. Exploring different modalities of growth factor inhibitor to treat cancer.[2,3] – Dr. Jon Elkins

Primary Supervisor: Dr. Jon Elkins
Second Supervisor: Prof. Valentine Macaulay
Eligibility: Track 2 and 3 students are eligible to apply for this project.

Abstract

Various cancers are promoted by the binding of growth factors to receptor proteins, and are also associated with higher levels of the growth factors. One strategy to treat cancer is to block the binding of the growth factor to the receptor. This can be done by a drug that binds to the receptor or by a drug that binds to the growth factor itself, such as bevacizumab which is a monoclonal antibody that binds the growth factor VEGFA and is used to treat multiple types of cancer. Antibodies can be discovered that bind to growth factors, but they are expensive to produce and require intravenous delivery. Cyclic peptide or small molecule inhibitors of growth factors could offer advantages over antibodies by being cheaper, less likely to be immunogenic and given orally. Many cyclic peptides have already been approved as drugs [1]. This project aims to accelerate the discovery of cyclic peptide and small molecule binders of growth factors, by examining the interfaces of antibodies and cyclic peptides when bound to them. The cyclic peptides discovered during the project may also become lead candidates in a drug discovery programme. The project will combine the discovery of cyclic peptides, the discovery of nanobodies (small single-chain antibodies that are very suitable for crystallisation), and the structural comparison of these cyclic peptides and nanobodies when bound to growth factors.

By comparing structures, we aim to identify the features of nanobodies that give strong binding to the growth factor, and apply that knowledge to the design of improved cyclic peptides. After an initial focus on insulin-like growth factor 1 (IGF1), for which materials are already available, the knowledge and process can be applied to other growth factors such as EGF or VEGFA. The output will be, for each growth factor studied, a cyclic peptide inhibitor and an understanding of the structural features necessary for drug design.

Research objective

General methodology -Cyclic peptides (CPs) will be selected in the Kawamura lab from large libraries by mRNA-display / peptide microarray technology, and the top enriched hits that bind to IGF1 but not to insulin will be synthesised. An initial set of CP binders of IGF1 has already been generated as a proof-of-concept. Later in the project other target proteins can be used as bait in the peptide selection experiments.

The top hits from the screens will be validated for IGF1 binding by biophysical assays such as Biolayer Interferometry (BLI), Surface Plasmon Resonance (SPR) or Isothermal Titration Calorimetry (ITC). The peptides with the highest binding affinity will be used in co-crystallisation studies.

In parallel in the Centre for Medicines Discovery a nanobody selection by ribosome display can be performed analogously to the selection of CPs. Top enriched nanobodies will be validated as for the CPs and also used for crystallisation studies.

Computational analysis of the structures will guide the design of improved CPs in subsequent iterations. Among the factors to consider in the analysis will be potency of binding, selectivity (over insulin in the case of CPs targeting IGF1) and physicochemical properties such as solubility and predicted stability in plasma. *Knowledge of how to generate potent CPs against growth factors will be a big step forward for the drug discovery community, and this knowledge could potentially be applied to small molecules as well.*

The most potent CPs with nanomolar affinity can be tested for blocking IGF1 signalling in cancer cell assays in the Macaulay lab. It is anticipated that the best CPs will form the basis of a translational drug discovery project

Background to the choice of IGF1 as an initial target - Insulin-like growth factors-1 and 2 (IGF1/2) are small proteins that are similar in sequence to insulin and are part of a system of signaling that cells use to interact with their environment. IGF1/2 proteins are secreted into serum and high levels of IGF1 in serum are causally associated with risk of developing prostate, breast and colorectal cancer and with prostate cancer mortality [2–5]. IGF1/2 are also implicated in the association of tall height with cancer incidence and high-grade prostate cancer [6,7].

The receptors for IGF1/2 are the IGF receptor (IGF1R) and insulin receptor (INSR-A). When the target cells are cancerous the effect of signals transmitted by the IGF receptor can be to promote tumour growth, invasion and resistance to cell death (apoptosis), which confers resistance to chemotherapy and radiotherapy [8]. Small molecule IGF1R inhibitors showed promise in the clinic but ultimately did not succeed as drugs, in some cases due to difficulty in inhibiting IGF1R without affecting the related INSR either directly, or indirectly via downregulation of IGF1R:INSR hybrid receptors, or because IGF1R inhibition caused hyperglycaemia [8].

An alternative strategy would be to target IGF1 or IGF2 directly, blocking (or neutralising) their interactions with the receptors. Boehringer Ingelheim have developed a monoclonal antibody, xentuzumab, that binds IGF1 and IGF2. Xentuzumab inhibits >90% of serum IGF bioactivity and has been tested in phase II clinical trials in breast and prostate cancer where it showed insufficient activity in unselected patients, although subgroups are potentially responsive [9]. A clinical trial in Oxford 'WINGMEN' (NCT05110495, Section 9) involving Macaulay may identify responsive subgroups. An orally-active drug is therefore urgently needed, in particular if WINGMEN results support trialing longer-term IGF blockade.

While a small-molecule IGF1 inhibitor would be ideal, IGF lacks a good binding site for a typical small molecule. Cyclic peptides possess the benefits of both antibodies and small molecules. CPs often show greater target affinity, bioactivity and biostability than their linear analogues, with advantageous properties over antibodies including synthetic tractability, low immunogenicity, cheaper production and potential for oral dosing, such as for cyclosporin. CPs can bind to their target with sub-nM affinity and >100-fold selectivity, even for challenging protein targets, including other growth factors such as HGF [10].

Team: The two supervisors on the project bring together expertise in structural biology and inhibitor discovery (Elkins) with expertise in cancer biology and IGF biology (Macaulay). This project is a collaboration with the lab of Prof. Akane Kawamura at Newcastle University, who is a world leading expert in cyclic peptide discovery by mRNA-display and peptide microarray technologies, and has a strong track record discovering cyclic peptide inhibitors of proteins of therapeutic interest.

Translational potential

The new cyclic peptides discovered may lead to clinical programmes, while the knowledge gained from analysis of binding motifs will accelerate the discovery of cyclic peptide based drugs.

Training opportunities

The student will experience a broad range of experimental science. In the Elkins lab the structural analysis of growth factor complexes will involve techniques such as molecular cloning, protein purification and crystallisation, X-ray structure determination and advanced biophysical measurements. In the Macaulay lab the various types of cellular assay necessary to evaluate the optimised cyclic peptides for their anti-cancer potential. The student will also have the opportunity to visit the Kawamura lab to learn about cyclic peptide discovery and synthesis. Overall, an education and training in structure-guided drug design in the cancer therapeutic area.

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48. The inactivation of p97 system in colorectal cancer therapy [1,2,3,4] – Prof Kristijan Ramadan

Primary Supervisor: Prof Kristijan Ramadan
Second Supervisor: Prof. Simon Leedham
Eligibility: All tracks are eligible to apply for this project.

Abstract
Colorectal carcinoma (**CRC**) currently represents the third most common type of cancer worldwide, accounting for approximately 10% of all diagnosed cases, with 1.85 million new cases annually [1]. The systemic treatment of microsatellite stable (MSS) colorectal cancers, which comprise around 80% of all cases, has remained largely unchanged since the early 2000s. Currently, two main therapies, FOLFIRI and FOLFOX, both exhibiting similar efficacy, continue to be the most commonly used first-line treatments, particularly for colon cancers [2, 3].FOLFIRI is a chemotherapy regimen based on irinotecan/topoisomerase inhibitor, which induces the formation of Topoisomerase 1 cleavage complexes (**TOP1ccs**), a specific type of enzymatic DNA-protein crosslink (DPC) where TOP1 is covalently bound to DNA [4]. In recent years, our research has highlighted the significance of p97 ATPase/unfoldase, SPRTN protease, and TEX264 in the repair of TOP1ccs and subsequent cell survival [5, 6]. TEX264, acting as a p97-cofactor, serves as the receptor for TOP1ccs. It binds to TOP1ccs and recruits the p97-SPRTN complex to the site of DNA damage (Fig. 1), facilitating the proteolytic degradation of TOP1ccs through p97 unfoldase activity and subsequent SPRTN protease activity. This mechanism allows cancer cell survival in response to TOP1 inhibitors.

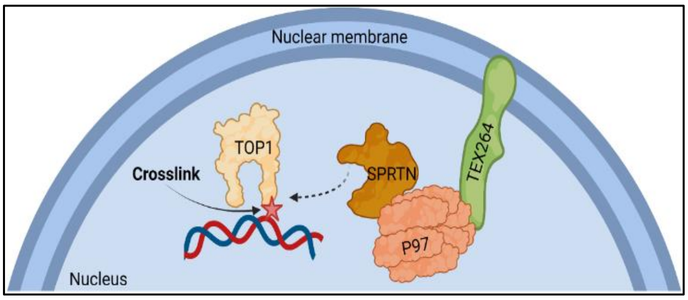

Figure 5. The role of p97-SPRTN- TEX264 machinery in repair/processing of cytotoxic TOP1ccs.

We have validated the importance of p97 ATPase, SPRTN protease and TEX264 cofactor in determining the efficacy of TOP1 inhibitors such as camptothecin and irinotecan in various human cancer cell lines in our laboratory (Fig. 2, data not shown), as well as in CRC patients (Fig. 3). Furthermore, we have established the p97-SPRTN-TEX264 complex as the first biomarker for predicting the response to FOLFIRI in CRC patients.

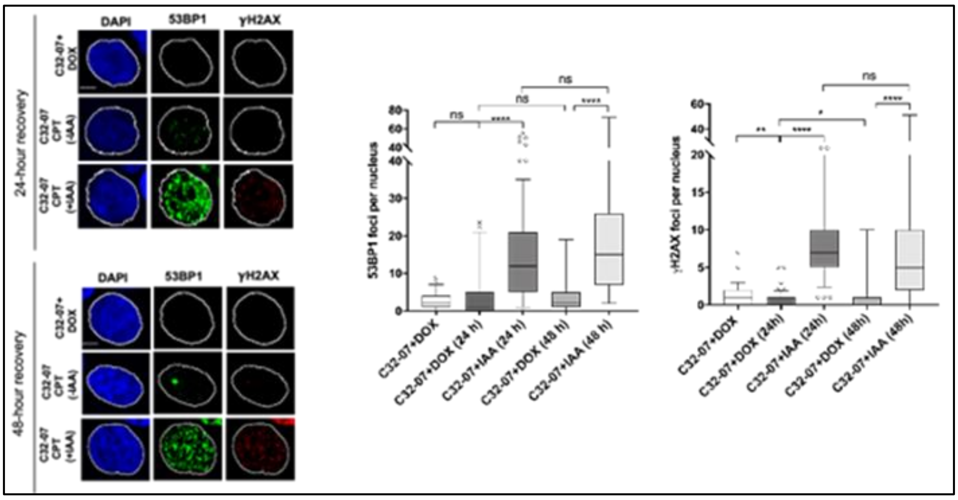

Figure 2. SPRTN depletion by auxin (IAA) inducible degradation increases the amount of DNA damage (γ-H2AX; 53BP1) in HCT116 cells treated with camptothecin (CPT) and delays its repair.

To inactivate this p97-SPRTN-TEX264 complex and thus kill cancer cells, we aim to use the second generation of orally available p97 inhibitor CB-5339 developed by our industry partner, Cleave Therapeutics. CB-5399 is currently undergoing clinical trials for various blood and solid malignancies, albeit not for colorectal cancer [7]. CB-5339 has successfully completed a phase I clinical toxicity study and is now progressing into phase II clinical trials.

With the support of Cleave Therapeutics (*by providing the inhibitor*), our objective is to investigate the molecular mechanisms of CB-5339 both *in vitro* and in an orthotopic CRC mouse model. This research will provide valuable insights into how p97, and thus the entire p97-SPRTN-TEX264 complex, contributes to the survival of CRC cells treated with CB-5339 alone or in combination with standard chemotherapies such as FOLFIRI and FOLFOX.

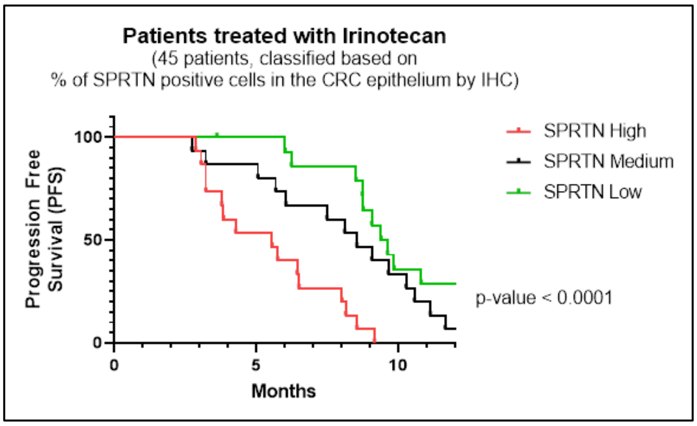

Figure 3. The overexpression of SPRTN correlates with a worse prognosis in patients treated with irinotecan.

Research objectives

- Characterise the sensitivity of different CRC cell lines to the p97 inhibitor CB-5339
- Investigate the role of the p97-SPRTN-TEX264 complex in the repairment of TOP1ccs induced by topoisomerase inhibitors in CRC with the focus on DNA replication and S-phase cell proliferation
- Validate our findings using CRC orthotopic mouse models treated with CB-5339 alone and in combination with FOLFIRI and FOLFOX.

Translational potential

A deeper understanding of the role of specialised machinery (97-SPRTN-TEX264 complex) for repair of cytotoxic TOP1-ccs in mediating the response to topoisomerase inhibitors will allow us to better predict the response to FOLFIRI in CRC patients. Moreover, p97 inhibition is likely to synergize with irinotecan-based chemotherapy to further improve the outcomes of patients treated with TOP1ccs-inducing drugs in many cancer types. This is a collaborative work between, basic science (K. Ramadan), pre-clinical validation (S. Leedham) and industry partner (Cleave Therapeutics; a support letter can be provided).

Training opportunities

The PhD student will have the opportunity to get trained in basic science (molecular, cell biology and rodent work), pre-clinical research (mouse work) and understand how to translate a clinical problem (chemotherapy resistance) to the bench and return this knowledge back to the clinics.

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49. Assessment of Oesophageal cancer patient responses to immunotherapy via human tissue avatars.[1,2,3] – Prof. Richard Owen

Primary Supervisor: Prof. Richard Owen
Second Supervisor: Prof. Eric O'Neil
Eligibility: Track 1,2 and 3 students only are eligible to apply for this project.

Abstract
Currently, the majority of in vitro therapeutic testing is carried out using patient-derived cell lines, xenografts (PDX) and genetically engineered mouse models (GEMMs). While murine models provide valuable information about the systemic effects of therapeutics, GEMMs fail to capture the genomic heterogeneity of native tumours and PDX models are challenging to establish orthotopically and do not allow for the interaction of tumour with a functional immune system to be studied (Kim et al., 2009). Introduction of patient- derived organoids has allowed in vitro analysis of treatment interactions with three-dimensional tumour structures (Ooft et al., 2019) but, similarly to xenograft models, frequently fails to capture the complexity of the TME (Larsen et al., 2021). Recent efforts move in the direction of incorporating multiple different cell types and vascular mimics to reconstruct tumour complexities (Neal et al., 2018). Patient-derived tumour slices provide a platform through which tumour, stroma and immune infiltrate can be studied in their native architecture (Ghaderi et al., 2020; Kokkinos et al., 2021). Through this system therapeutics can be investigated for their impact throughout the tumour, allowing analysis of intra-patient variation in a clinically relevant timeframe.

O'Neill lab has developed the use of live patient-derived tumour slices for dissection of pancreatic cancer microenvironment and investigation of therapy responses. Methods have established to maintain superior cellular fitness and preservation of tumour microenvironment compared to standard cultures, organoids or spheroids. Analysis of transcriptomic changes induced by a combination of therapies aimed to target metabolic reprogramming treatment shows the potential of the platform to interrogate treatment responses across all cellular compartments of the microenvironment, in particular immune, in an unprecedented manner. Having demonstrated that organotypic tumour slices can maintain viability and provide novel insights enhancing both novel therapeutic discovery and precision medicine to improve current standard of care.

Oesophageal cancer is the sixth leading cause of cancer mortality worldwide (Bray et al., 2018). The predominant subtype in the western world, oesophageal adenocarcinoma, is among the cancer types with the highest increase in incidence over the past few decades (Devesa et al., 1998; Fitzgerald, 2004; Groulx et al., 2020; Lepage et al., 2008; Pennathur et al., 2013). About 40% of oesophageal cancers present with distant metastases at diagnosis (Smyth et al., 2017) and for these inoperable patients, median overall survival (OS) with conventional agents is less than one year (Cunningham et al., 2008; Dijksterhuis et al., 2019; Janmaat et al., 2017; Jatoi et al., 2006; Waddell et al., 2013). Treatment regimens using αPD-1 with chemotherapy have been approved and an Oxford-based trial (LUD2015-005) recently performed comprehensive clinical and molecular profiling throughout treatment using a combination of whole genome sequencing (WGS), single-cell RNA-sequencing (scRNA- seq), and bulk RNA-sequencing (bulk RNA-seq) to identify patients that benefit. Treatment-responsive molecular signatures were identified that effectively predict response and resistance to first-line αPD-1 and also predicted long-term αPD-1 outcomes in other settings (Carroll et al. *in press*). Notably, high PD-L1 expression and tumour mutational burden composed indicators to establish pre-treatment biomarkers that could improve prediction of long-term outcomes of αPD-1 treatment.

Research objective
This project is aimed to develop live tissue patient avatars from oesophageal adenocarcinoma biopsies using the technology validated for immune monitoring of pancreatic cancer avatars in the O'Neill lab. We aim to use engineer this approach to screen for patients susceptible to αPD-1 therapy and a platform to assess further immune-therapies as potential combinations for patients not served by the pre-treatment biomarkers I have found (Carroll et al. *in press*).

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50. The T-cell receptor landscape of adult diffuse gliomas, a non-invasive tool for tumour detection and classification? [1,2,3,4] – Assoc Prof. Olaf Ansorge

Primary Supervisor: Assoc Prof. Olaf Ansorge
Second Supervisor: Dr. Bo Sun
Eligibility: All tracks are eligible to apply for this project.

Abstract
Adult diffuse gliomas are the most common malignant tumours affecting the central nervous system[1]. Glioblastoma multiforme (GBM) is the most common and aggressive form. It affects up to 18 people per 100,000 and has a poor survival time of approximately 12-18 months. Although diagnostic scans and treatments have advanced, there is still a significant need for non-invasive and reliable methods to detect and monitor gliomas. Indeed, current imaging-based methods are often insensitive to early tumour occurrence/recurrence[2,3], and biopsies carry significant neurologic risks. To address this critical unmet need, our interdisciplinary study aims to evaluate the potential of T cell receptor (TCR) sequencing approaches as a non-invasive biomarker for early detection and monitoring of adult diffuse gliomas.

T-cell receptor sequencing captures intra-tumoural T-cell responses
T-cell receptor (TCR) sequencing shows promise for early cancer detection and monitoring in various cancer types, including GBM[4-8]. T-cells play a crucial role in the adaptive immune system and their involvement in cancer is increasingly recognised. TCR sequencing of peripheral blood is a sensitive method for detecting the clonal evolution of intra-tumoural T-cell populations, which has been associated with better prognosis[4-6]. In glioblastoma, recent studies have shown that TCR repertoires in blood can serve as a sensitive biomarker of the intra-tumoural T-cell response, despite the brain's perceived immune privilege[6-7].

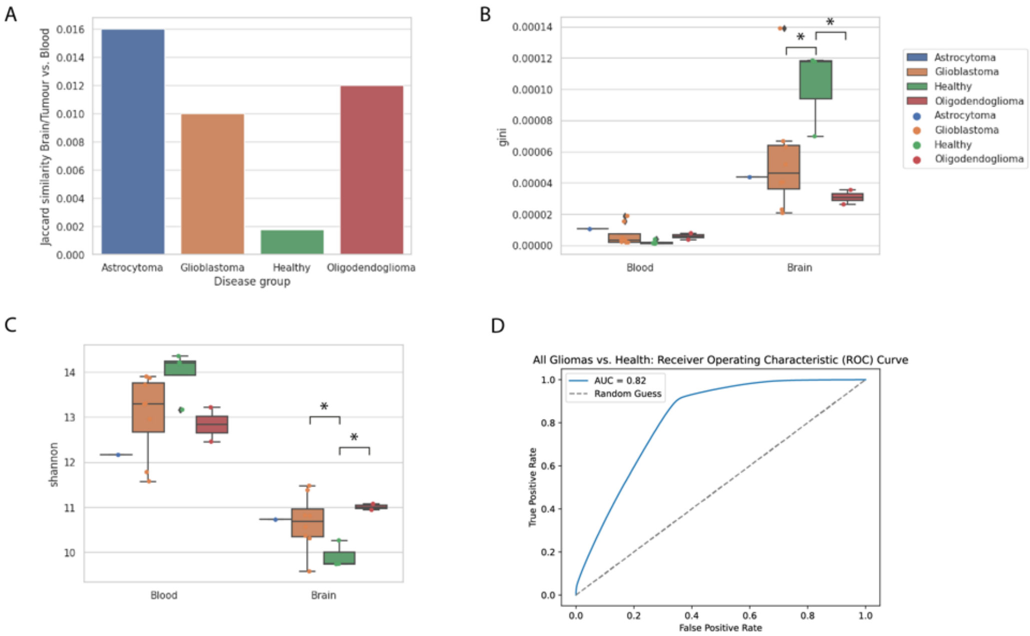

Figure 1. Re-analysis of Sims et al., PNAS, 2016. (A) Jaccard similarity indices were calculated across blood derived TCRs vs. tumour/brain derived TCRs per condition. Higher Jaccard indices indicate more similarity in the repertoires. (B) Gini coefficients were calculated across study groups within anatomical sites. Each point denotes an individual sample. P values denote within-group post-hoc dunn tests; * = <0.05. (C) Shannon's entropy was calculated across study groups within anatomical sites. Each point denotes an individual sample. P values denote within-group post-hoc dunn tests; * = <0.05. (D) Receiver operating characteristic curve analysis results of a two-layer 2D Convolutional neural network feed-forward model. Dotted line denotes the true and false positive rates from random

Machine learning demonstrates feasibility in disease TCR repertoire classification tasks
Our own re-analysis of a published glioma TCR sequencing dataset[8] lends support to the feasibility of the proposed research by revealing disease-specific TCR signatures in patients with adult diffuse glioma. We observed a greater clonal overlap between matched tumour/brain and blood samples in adult diffuse glioma patients compared to healthy donors (Figure 1A). Additionally, we observed decreased Gini coefficients and increased Shannon's diversity indices, supporting tumour-specific disturbances of TCR clonality (Figure 1B, C). To demonstrate feasibility of TCR-guided disease classification, we trained a deep learning model on one-hot encoded CDR3 amino acid sequences of TCRs, which achieved an area under the receiver operating characteristic (AUC) curve score of 0.82 (Figure 1D).

Research objective

The proposed project aims to investigate the utility of TCR sequencing in early detection and monitoring of gliomas using both established short-read and novel long-read sequencing approaches. The overall objective is to evaluate the ability of TCR sequencing to detect and monitor immune responses in patients with adult diffuse gliomas. To achieve this, the study has three primary objectives:

1. To perform cross-sectional short-read and long-read TCR sequencing of paired tumour and blood samples from patients with adult diffuse glioma, and matched controls.
    a. Paired tumour and blood samples will be derived from the established Tessa Jowell Brain Matrix project (UK).
2. Evaluate and compare the reproducibility of long-read nanopore TCR sequencing to the previously established short-read approach.

3. Use machine learning models, such as deep neural networks, support vector machine and variational autoencoders to identify peripheral TCR repertoire patterns that predict the intra-tumoural T-cell response, and correlate these to tumour mutation burden, and glioma molecular subtype.
    b. Computational training and support will be provided by co-supervisor Dr Bo Sun in collaboration with Associate Prof. Rachael Bashford-Rogers.

Translational potential

The proposed research has the potential to significantly impact patient care by providing a non-invasive and reliable method for early detection and monitoring of glioblastoma and other diffuse gliomas. By identifying tumour-specific T-cell receptor (TCR) repertoire patterns and developing machine learning models to predict the presence and molecular subtype of cancer cells, this research may enable clinicians to detect tumours at an earlier stage and track tumour recurrence post-surgical intervention. This is particularly pertinent in light of the active development of immune therapies in neuro-oncology such as CAR T-cells. Furthermore, recent FDA authorisation of the T-detect COVID test[9], a TCR sequencing-based diagnostic tool for identifying infected individuals, is evidence of the clinical feasibility of TCR-based approaches. Its approval paves the way for the development of TCR-based approaches for use in clinical settings.

Training opportunities

Where appropriate, the student will receive training in Good Clinical Practice principles and the conduct of observational studies. From a laboratory perspective, flow cytometry, cell sorting, RT-PCR and library preparation of both short-read and long-read sequencing modalities will be used in this project. Hands-on immunology experience and training in T cell receptor enrichment and library preparations will be provided. For bioinformatics training, the student will be supported to attend dedicated computational course offered under the wider network of the Medical Sciences Division; further, direct supervision will be provided by Dr Bo Sun. The student will have full access to the facilities, expertise, and resources available within NDCN, the department of Neuropathology and across the broader community at the University of Oxford. By the end of the project, the candidate will be in a strong position to drive fundamental and translational clinical research in neuro immuno-oncology and adaptive receptor repertoire sequencing.

References

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51. Take epigenetic diagnostics "from bench to bedside"[1,2,3,4] –Prof. Prof Benjamin Schuster-Böckler

Primary Supervisor: Prof Benjamin Schuster-Böckler
Second Supervisor: Assoc Prof. Ben Fairfax
Eligibility: All tracks are eligible to apply for this project.

Abstract
Cancer initiation and progression is not solely driven by mutations: it is becoming increasingly clear that environmental factors, differentiation dynamics and inflammation put cells in a state that is more (or less) susceptible to cancerous transformation. Such dynamic cell states are maintained by cells through epigenetic marks on histones and DNA. We have a particular interest in decoding the role of changes in the DNA methylation in cancer formation and progression.

Together with the group of Prof Song, we previously developed "TAPS" (TET Assisted Pyridine Borane Sequencing), a new sequencing method for measuring methylated cytosine in DNA that produces much cleaner data from low-input samples such as cell-free or single-cell DNA[1]. We recently used TAPS to generate a base-resolution atlas of tissue-specific methylation and hydroxymethylation from over 20 different cell and tissue types, as well as from a range of different cancer types. This map has revealed a whole range of differentially methylated regions that uniquely identify cell types and show dysregulation in cancer.

We are involved in several clinical trials that collect whole-genome TAPS data from cell-free DNA of patients at risk of or diagnosed with cancer. For example, we collaborate with Prof Lu and Dr Owen on a trial of checkpoint inhibition in oesophageal adenocarcinoma[2], as part of which we are collecting cell-free DNA methylation data of nearly 100 patients. **The goal of this DPhil project will be to leverage cell-type and tissue-specific epigenetic information to detect whether patients have cancer and/or whether they will respond to immunotherapy[3].**

Research objective
Objective 1: Predict cancer immune landscape from ctDNA

In our recent Cancer Cell paper[2], we demonstrate that mutation burden and monocyte content of the tumour at diagnosis are independent predictive biomarkers of immune-checkpoint therapy success in oesophageal adenocarcinoma. Immune cells are the main contributors to cell-free DNA in healthy individuals as well as cancer patients. The first objective of the DPhil project would be to determine whether the contribution of T-cell, macrophage and monocyte DNA varies between healthy donors and cancer patients, and whether we can identify changes in the regulatory regions that control e.g. monocyte specific marker genes that we identified in our previous RNA-seq analysis. **The proposed outcomes of this work will be a method to quantify the immune-cell contribution in cell-free DNA based on epigenetic marks.**

Objective 2: Quantify mutation burden from ctDNA

We are currently working on methods to improve somatic variant prediction from cell-free TAPS data using machine-learning to improve the signal-to-noise ratio[4]. Using such pre-processed data, we will test whether ctDNA derived mutation burden correlates with mutation burden measurements derived from whole-genome sequencing of the same patient's tumour. **The expected outcome of this work will be a method to quantify tumour mutation burden based on cell-free DNA TAPS data, and an evaluation of the accuracy of these predictions.**

Objective 3: develop an integrated predictor of treatment success

Finally, we hope to combine the results of objective 1 and 2 into an integrated classifier that combines mutation burden and cell-composition estimated from cfDNA to predict patient outcome. We will evaluate the performance of this classifier on an independent dataset of patients treated with neo-adjuvant immunotherapy. **The expected outcome will be a quantitative evaluation of prediction accuracy, which will be fundamental for securing further collaborations to translate this work into a clinically useful tool.**

Translational potential
This is a highly translational project by design. Any biomarkers and predictive algorithms developed as part of this project will be applied first within existing clinical trials and, if promising, would form the basis for industry collaborations to test these approaches in larger cohorts.

Training opportunities
This project would be an ideal opportunity for a candidate with a passion for epigenetics and an interest in computational biology. You will learn how to process and analyse DNA methylation and other epigenetic data using statistical tools. Some experience with R or Python and basic linux command line usage would be helpful.

Initially, this project will be very "data science" heavy, ie identifying correlations and associations between linked datasets. Further down the line, you will also learn to build predictive algorithms, apply them to data and evaluate their performance.

References

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52. Spatial interrogation of low grade prostate cancer to identify genomic events responsible for driving indolent not aggressive disease[1,2,3,4] – Dr. Alistair Lamb

Primary Supervisor: Dr Alistair Lamb
Second Supervisor: Prof. Ian Mills
Eligibility: All tracks are eligible to apply for this project.

Abstract
Background: Over the past decade we have increasingly accepted that prostate cancer classified as "low risk" (Gleason Grade Group 1 (Gleason Score 3+3 = 6), PSA <10, Stage T1-T2a) are indolent and do not progress.[1] This has led to a recommendation for most men with this type of prostate cancer to undergo active surveillance.[2 3] Indeed, opinion leaders in prostate cancer are increasingly calling for Grade Group 1 prostate cancer to cease being called "carcinoma".[4 5] However, we know little about the genetic composition of such low grade tumours, largely because research has focussed on higher grade disease, but also because we have lacked the spatial genomic technologies to carefully interrogate discrete selected areas of prostate tissue. Those technologies are now available[6 7] and have been developed to a point where the clonal copy number composition of epithelial regions can be defined,[7 8] although they still lack spatial epigenomic analyses.

In our organscale study of a prostate removed at radical prostatectomy which had multifocal prostate cancer, we identified an area of Gleason Grade Group 1 prostate cancer which lacked most of the defining somatic mutations of higher grade disease (e.g. chr8q gain corresponding to well-known prostate cancer oncogene c-Myc[9], or chr10p loss corresponding to tumour suppressor gene PTEN[10]; **Figure 1**). This raises the possibility that low grade cancer is fundamentally distinct from higher grade disease at a genetic level. We also found that areas of non-transformed 'benign' epithelia contained areas of greater genomic risk than low grade cancer. Perhaps low grade cancer is therefore 'safer' than benign epithelia which still retains the potential to undergo somatic mutations that develop aggressive disease.

Questions: Why do low grade Gleason pattern 3 prostate cancers seem not to progress? How can we better differentiate "good" from "bad" pattern 4 disease? Can we drive "bad" pattern 4 disease to indolence?

Hypothesis: Low grade prostate cancer clones harbour genomic alterations which pre-determine indolence; when present in higher grade clones, such alterations differentiate high grade disease with good prognosis.

Research objectives

1. Consolidate: To undertake clonal siCNV analysis[7] on a selection of men with pure Gleason pattern 3 prostate cancer (n=5; from archive as we no longer operate on these men) extending the findings of our recent paper in Nature, Erickson et al[7], to identify "indolence factors" when benchmarked against pattern 4 & 5 [tissue handling; spatial transcriptomics; bioinformatics]

2. Extend: To track clonal composition of Gleason pattern 3 and 4 cancer in serially sampled MRI-targeted biopsies from men on active surveillance for "low" and "intermediate risk" prostate cancer (n=5 each) [spatial transcriptomics; DNA sequencing; epigenomics; bioinformatics]

3. Establish phenotype: To genetically modify cell-line models of aggressive PCa (e.g. PC3, DUCaP) to upregulate indolence factors as a pre-clinical basis for pharmacological manipulation [cell culture; cloning; lentiviral transduction; functional assays]

Translational potential
The data produced could provide the evidence our field is looking for to underpin a redefining of low grade prostate cancer, as well as helping us understand what makes some pattern 4 prostate cancers so much worse than others. This could help a proportion of men who currently undergo radical therapy (currently approx. 25,000 pa in UK) to avoid the side-effects of such treatment.

Training opportunities

The project incorporates a range of wet and dry lab techniques (see [] brackets above) and can be tailored, given our overall lab skill sets, to someone with an interest in biology or computational work. We will also train the individual, whatever their background, in our overall approach to translational surgical science with opportunities to work with surgical oncologists, pathologists and radiologists as well as biologists and data scientists. https://www.nds.ox.ac.uk/research/prostate-biology

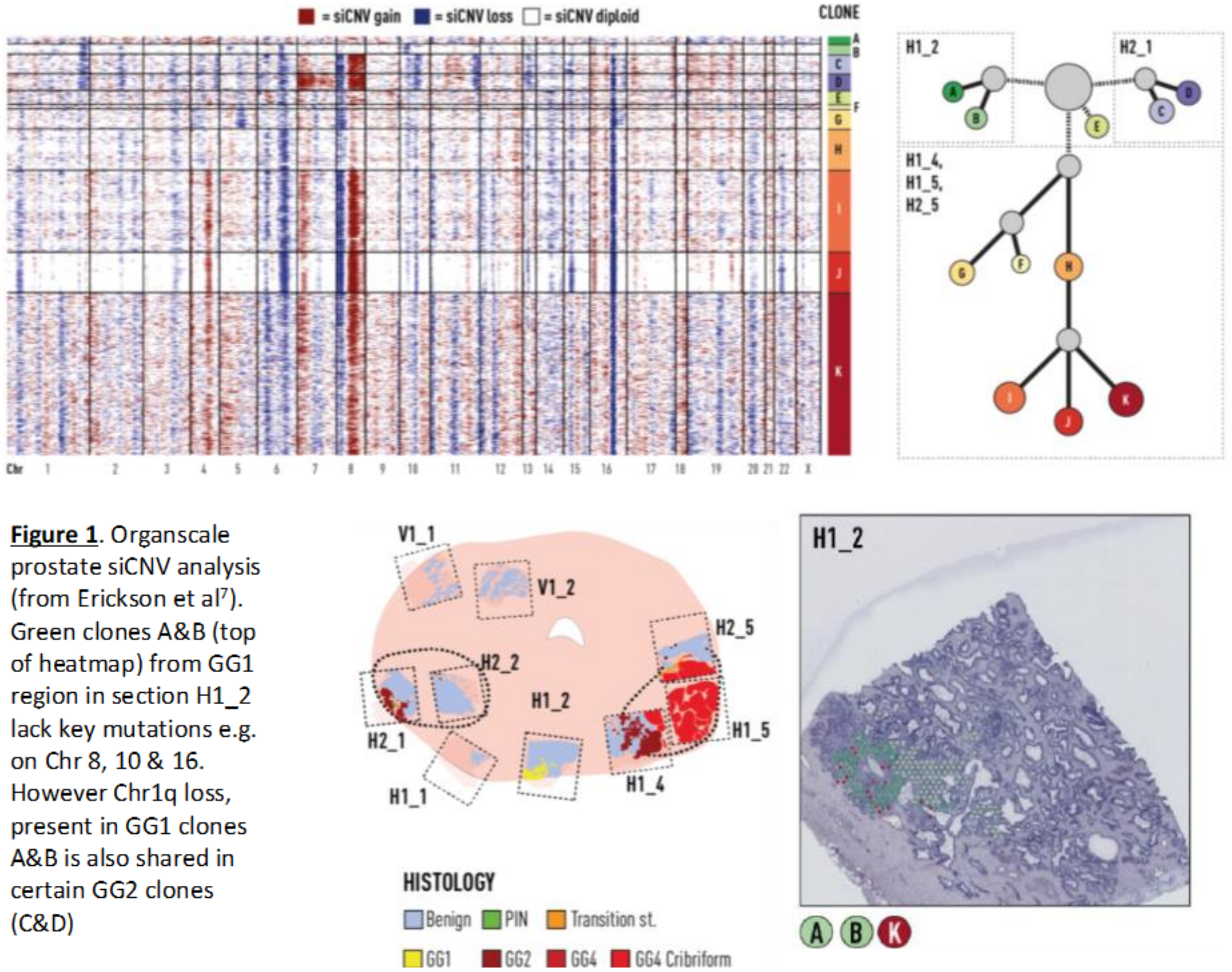

Figure 1. Organscale prostate siCNV analysis (from Erickson et al[7]). Green clones A&B (top of heatmap) from GG1 region in section H1_2 lack key mutations e.g. on Chr 8, 10 & 16. However Chr1q loss, present in GG1 clones A&B is also shared in certain GG2 clones (C&D)

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53. New Immune Therapies for Acute Myeloid Leukaemia (AML) And Myeloid Blood Cancers [1,2,3,4] – Prof. Paresh Vyas

Primary Supervisor: Prof. Paresh Vyas
Second Supervisor: Dr, Ricardo Fernandes, Prof. Omer Dushek and Professor Gillespie.
Eligibility: All tracks are eligible to apply for this project.

Abstract
Immunotherapy is transforming cancer outcomes. This project focuses on developing a mechanistic understanding for developing new T cell receptor (TCR) based treatments for AML, the most common aggressive adult blood cancer. We have identified peptides bound to HLA class II and T cell receptors that recognise these peptides by studying AML patients cured by allogeneic stem cell transplantation. Though many groups are working on developing HLA-I restricted therapies, very little is known about how to harness HLA-II therapies in cancer. To address this important knowledge gap, the project will study the mechanisms that govern binding of peptide to HLA-II and the binding of TCR to peptide-HLA-II (pHLA) antigen. This is a pre-requisite to developing effective HLA-II directed TCR-based therapies. The applicant will work in a multi-disciplinary group of immunologists, AML clinical academics and structural biologists to transform immune therapy for AML and related myeloid cancers. There are three discrete packages of work. First, experimental and computational approaches will be employed to study HLA-II peptide binding. Second, experimental approaches will study the binding and crossreactivity of TCRs to pHLA-II antigens. Finally, the applicant will collaborate with a structural biology group to study the structural basis of p-HLA-II-TCR binding.

Research objectives
Background: Over the last few years, we have been working to understand the mechanistic basis of cure mediated by the most established curative cellular immunotherapy, allogeneic stem and immune cell transplantation (allo-SCT). Allo-SCT involves transfer of blood stem and immune cells from a healthy person (donor) to a patient (recipient) (Fig. 1a). Allo-SCT has been in routine clinical practice since the 1960s and around 20,000 allo-SCTs are performed worldwide annually. The most common disease treated by allo-SCT is AML. Allo-SCT is curative because some of the donor immune cells, called T cells, attack and eradicate the patient's cancer cells. This is called Graft-versus-Leukaemia (GvL). However, donor immune cells can also attack the patient's normal healthy tissue, which can cause great harm. This is known as Graft-versus-Host Disease (GvHD) (Fig 1b).

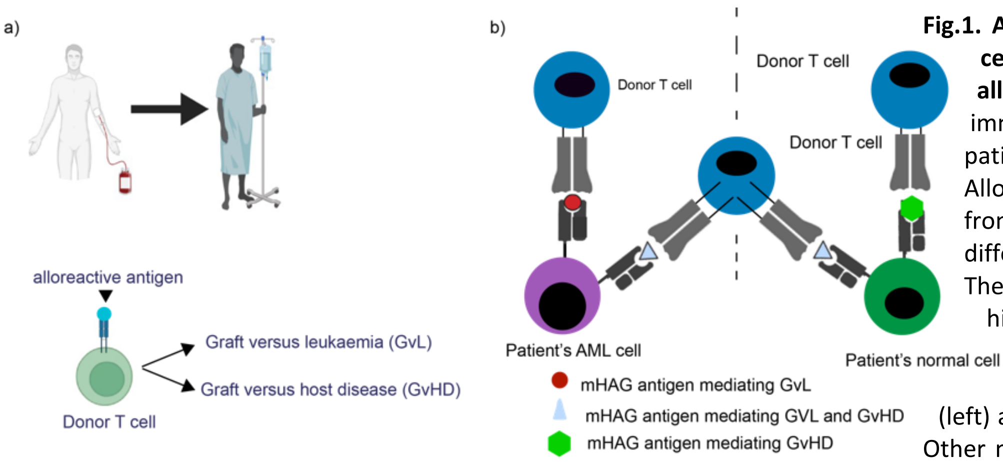

Fig.1. Alloreactive antigen-specific T cells mediate GvL and GVHD in allo-SCT. a) Donor (left) stem and immune cells are infused into a patient after chemotherapy. b) Alloreactive antigens mainly arise from coding germline SNPs that differ from patient and donor. These are known as minor histocompatibility antigens (mHAG). Some mHAGs are presented only on AML cells (left) and elicit GvL T cell responses. Other mHAGs are expressed only on normal tissue and elicit GvHD (right). Finally, some mHAGs are expressed on both AML and normal cells and elicit both GvL and GvHD

Remarkably, until our recent work, the field has limited insight into the antigenic specificity of GvL, or how to distinguish T cells that cause beneficial GvL from those that cause harmful GvHD. To address this knowledge deficit, we used an unbiased systematic reverse immunology approach to study elite responders who had been cured by allo-SCT, with minimal GvHD. We identified 22 peptide antigens targeted by alloreactive T cells due to single amino acid differences between patient and donor encoded by **germline** single nucleotide polymorphisms (SNPs) that differed between patient and donor, rather than somatic mutations in the patient's tumour (Fig. 2).

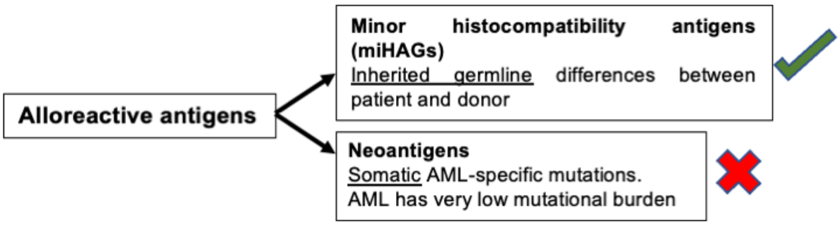

Fig. 2. Alloreactive antigens. In principle these could be either mHAGs (due to coding germline SNPs) or neoantigens (due to somatic AML-specific mutations. Our data shows the antigens identified were mHAGs consistent with the low mutational burden in AML.

1. Characterisation of HLA-II binding to alloreactive peptide Using molecular biology and protein engineering methods the applicant will express soluble pHLA-II with varying peptide lengths and study the biophysical properties of peptide-HLA-II binding and compare this to computationally derived prediction of peptide-HLA-II binding. By mutating peptide resides the applicant can identify which peptide residues are critical for binding. This work will be combined with structural studies in Aim 3.

2. Characterisation of TCR binding and crossreactivity to pHLA-II antigen. Using molecular biology and protein engineering methods the applicant will express soluble TCR and test the biophysical properties of binding to pHLA-II to TCR. By mutating TCR resides the applicant can identify which TCR residues are critical for binding. If time permits, the applicant will generate a large, unbiased, peptide-MHC library for yeast display to determine the TCR crossreactivity of wild-type and engineered TCRs. Finally, for select TCRs that bind pHLA-II the mode of T cell activation and need for co-stimulation will be studied. This work will be combined with structural studies in Aim 3.

3. *Structural studies of TCR-PHLA-II binding* The data in Aims 2 and 3 will be greatly strengthened by structural studies of pHLA-TCR interaction. The applicant will take select soluble p-HLA-II antigens and cognate TCRs from aims 2 and 3 collaborate with a structural biology group to enable them to make crystals for structural studies using a range of structural resolution methods.

The combination of Aims 1-3 will provide detailed insight into the mechanisms of p-HLA-II binding and the binding of pHLA-II antigens with TCRs.

Translational potential
This project aims to identify the optimal pHLA antigens and TCRs for either TCR engager therapy or TCR T-cell therapies for AML and myeloid cancers.

Training opportunities
The DPhil student will be trained in: (i) fundamental aspects of immunology and specifically pHLA interactions and the binding of pHLA to TCR; (2) molecular biology, protein engineering and biophysical measurements of protein-protein interaction: (3) computational biology (4) structural biology. The training will be focussed on specific skill sets that are critical for developing immune therapies.

References:
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54. Mapping the ovarian cancer ascites ecosytem for discovering novel therapeutic approaches [1,2,3] – Dr. Nicola Ternette

Primary Supervisor: Dr. Nicola Ternette
Second Supervisor: Eleni Adamopoulou and Prof Tim Elliott
Eligibility: Track 1, 2 and 3 students are eligible to apply for this project.

Abstract
Dynamic interactions between cancer, immune and stromal cells of the tumour microenvironment are critical for tumour development and contribute to treatment resistance. However, a systematic molecular and cellular understanding of how these interactions change during tumour progression, treatment, and their association with clinical prognosis is still lacking. For the most part, this is due to limited sampling and cell availability of primary tumour tissues for extensive studies. Ovarian cancer (OC) is the most lethal of gynaecological cancers largely due to its late diagnosis, high metastatic potential, and resistance to chemotherapy. Almost all advanced stage OC patients develop malignant ascites[1]. Cancer-associated ascites is enriched for tumour, immune, and stromal cells, creating a unique local tumour-immune microenvironment (TIME) that could be used as a surrogate resource for immune cell monitoring. However, the extent that ascites reflects the primary tumour tissue microenvironment remains unknown. The aim of this project is to investigate how the TIME in the ascites of OC shape tumour progression over time and dissect the effect of treatment on immune and tumour cells associated with better prognosis. This will be conducted by integrating deep cell phenotyping, multiplex spatial profiling and mass spectrometry (MS)-based immunopeptidomics for uncovering unique and common features of the TIME of primary tumours and ascites during disease progression and response to therapy, thereby determining predictors of good prognosis. Leveraging network-based analysis, this approach has broad potential for informing the design of more effective therapeutic strategies.

Research objective

1.High-dimensional phenotyping of the TIME in ascites from advanced-stage OC patients. To comprehensively measure the trajectory of interactions between immune and tumour cells prior to and during chemotherapy, a multiplexed immunofluorescence approach for cells isolated from ascites will be used. This will be complemented by *in-vitro* functional assays in cross-sectional studies. In collaboration with key experts we have developed and validated 35-colour spectral flow cytometry panels for high-throughput protein-based screening of tumour, stromal, T-, B-, NK- and myeloid cells. Preliminary interrogation of ascites pre- and post-chemotherapy revealed complex immune cell profiles with a good representation of cell subsets compared to those observed in peripheral blood mononuclear cells from healthy individuals. Chemotherapy results in redistribution of T-cell subsets, increasing PD-1$^+$ non-cytotoxic CD8$^+$ T cells. Given the role of tumour-specific CD8$^+$ cytotoxic cells in clearing tumour cells, this suggests a potential clinical advantage of using immune checkpoint blockade therapies to rescue this crucial cell subset. However, immune checkpoint blockade in ovarian cancer patients has to date not proven beneficial to disease outcomes[2]. Our study aims to identify factors driving this lack of response and potential new targets and/or combinations. Furthermore, tumor cells are phenotypically heterogeneic with specific subsets dominating the ascites ecosystem after extensive chemotherapy. This heterogeneity can have important implications for various aspects of cancer, including tumor growth, response to treatment, and the development of resistance. We aim to gain a deeper view of the different subsets of tumour cells with unique characteristics and properties. Our preliminary findings support the hypothesis that the composition of ascites represents the TIME and supports its use as a relatively non-invasive source of patient material that could yield new predictive and prognostic biomarkers. We aim to profile a large number of ascites samples from well-stratified OC patients and (a) identify correlations between ascitic immune-tumour profiles and treatment outcomes with a view to establishing tractable mechanistic hypotheses using deep immunophenotyping and (b) compare the composition of ascites to that of the primary tumour microenvironment by employing multiplex spatial profiling in a subset of patients where matched primary tissue samples are available, extending our multiplex panels to include other immune and stromal cells[3]. **Outcomes:** These data will help to define the functional immune-stromal-tumour cell interactions occurring in ascites contributing to tumour clearance and chemoresistance. It will enable the development of a Tumour Immune Cell Atlas compendium in ascites of OC that will serve as a reference model for studies in other cancer types like colorectal and pancreatic and aid in the development of innovative therapeutic approaches.

2. Exploring the tumour cell immunopeptidome. Characterisation of tumour antigens is essential for making the link between T cell specificity and therapeutic efficacy, and for the design of safe and effective cancer vaccines. Mass spectrometry (MS)-based immunopeptidomics is currently the only method that enables the direct identification of MHC-bound peptide antigens but is limited by the volume of cells required and also unable to assign candidate targets to cell subtypes within the tumour mass. Ascites represents a great alternative source for tumour cells in sufficient numbers for deep immunopeptidome profiling and in a state (suspension) that is amenable to bulk sorting prior to Immunopeptidomics. Leveraging our synergistic expertise in antigen processing/presentation, cutting edge MS-based immunopeptidomics and antigen discovery [4-6], we will employ deep immunopeptidome analysis of flow-sorted tumour cell subsets from ascites samples collected pre- and post-chemotherapy. Tumour cell signatures identified in Objective 1 will be used to select the most appropriate surface markers for the isolation of representative tumour cell populations for novel antigen target discovery. **Outcomes:** These data will provide a comprehensive characterization of the antigenic landscape of OC, enabling better-informed immunotherapies and facilitating the development of next-generation cancer vaccines.

Translational potential

In-depth patient sample profiling has direct translational potential. Ascitic fluid is an underutilised but unique biospecimen for research due to easy accessibility, successive sampling, and capacity for capturing changes in the cell populations that compose the TIME. Furthermore, deep knowledge of the tumour cell immunopeptidome could better inform immunotherapies and help develop the next-generation cancer vaccines. This study will support further translational studies to investigate diagnostic, therapeutic, and prognostic markers.

Training opportunities

Training on cell culture, spectral flow cytometry, MS-based immunepeptidomics, multiplex immunofluorescence, digital spatial profiling, bioinformatics techniques for data analysis will be provided.

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55. Elucidating the role of trans-lesion synthesis DNA polymerases in mutational processes and therapy resistance[1,2,3,4] - Marketa Tomkova

Primary Supervisor: Marketa Tomkova
Second Supervisor: Prof. Ian Tomlinson
Eligibility: All tracks are eligible to apply for this project.

Abstract

Trans-lesion synthesis polymerases (TLS) enable cells to replicate damaged DNA that would otherwise lead to replication fork collapse and cell death. However, TLS polymerases are inherently error-prone and introduce new mutations into the DNA, potentially contributing to the development of cancer. Understanding the sources and mechanisms of cancer-causing mutagenesis is critical for identifying effective preventive strategies, predicting personalised response to therapy, and designing novel therapeutics. One of the open questions in cancer mutagenesis is what proportion of the cancer-causing mutations are due to errors made by DNA polymerases during DNA replication.[1,2] The aim of this project is to elucidate the role of the error-prone TLS polymerases in mutational processes, using computational genomics combined with wet-lab approaches. Second, we aim to develop computational tools predictive of response to therapy, as TLS polymerases contribute to resistance to chemotherapy by bypassing replication-blocking lesions induced by chemotherapy such as cisplatin[3–5].

Research objective

Aim 1: Computational genomics approach to identify mutational signatures of TLS polymerases

Carcinogens and mutagenic processes leave distinct footprints in DNA, detectable using the computational approach of *mutational signatures*[6]. Remarkably, aetiology of nearly one-third of the mutational signatures in cancer patients is unknown, and there are open questions about the exact molecular mechanisms in many of the described mutational signatures.[7] Understanding these mechanisms is important for prevention (e.g., to know how we can change our lifestyle to avoid cancer), predicting risk and personalised therapy (e.g., using the signatures as biomarkers), and designing novel therapeutics (e.g., based on synthetic lethality).

The first aim of this project is to identify the contribution of TLS polymerases to the previously detected mutational signatures and to develop refined TLS signatures using novel computational approaches by utilising additional genomic features and other data (including TLS gene expression, locations of regions where TLS polymerases get recruited, and specialised datasets of samples deficient in one TLS polymerase and compensated by other TLS polymerases). Candidate mutational signatures of TLS polymerases will be identified, comparing traditional ways of de novo signature extraction, with novel approaches, such as deep-learning-based methods.

Aim 2: Direct *in vitro* and *in vivo* measurement of error-signatures of TLS polymerases

One of the challenges in studying DNA polymerase errors is that they are very hard to measure. We have recently developed a specialised technique called Polymerase Error Sequencing (PER-seq) to detect the errors made by DNA polymerases in single molecules *in vitro* (cell-free) in unprecedented detail. Here, we will apply PER-seq to selected TLS polymerases to obtain direct measurements of their error signatures, unobscured by DNA repair or other complex cellular processes. We will then complement this with sequencing of TLS-mutant/overexpression mouse and/or cellular models (Tomlinson lab) and analysis of sequencing data from other previously published resources.

Aim 3: Prediction of resistance to therapy due to TLS polymerases

Finally, we will evaluate the potential of these signatures to predict survival and resistance/response to treatment using data from cell-lines[9], recently cleaned and curated TCGA Resource[10], Genomics England, Hartwig Medical Foundation, ICGC and focussed datasets such as the SCOT clinical trial, and GDSC. Selected candidate predictions may be validated experimentally.

The expected outcomes of this project include (a) mutational signatures of TLS polymerases with support in human cancer data, *in vitro*, and *in vivo* models, (b) novel computational methods for signature detection, (c) mechanistic understanding of TLS role in mutagenesis, and (d) biomarkers of TLS-based therapy resistance.

Translational potential

TLS polymerases enable bypass of chemotherapy-induced DNA damage, leading to therapy resistance. TLS polymerases thus represent an attractive target for sensitizing cancer cells to genotoxic therapies. Indeed, inhibitors of TLS or their protein-protein interactions show promising synergy with therapies such as cisplatin, temozolomide, PARP inhibitors, and others [3–5,11,12]. It is thus of increasing importance to understand the mechanisms and extent of TLS contribution to chemoresistance and to develop biomarkers of resistance due to TLS polymerases. The signatures of individual TLS polymerases will elucidate which TLS polymerases are involved in resistance to different therapies, and will help to predict which patients would benefit from TLS inhibitor-based treatment. Finally, the aims 1 and 2 are also expected to elucidate the mutagenic role of TLS polymerases in genesis of different cancer types, with potential implications for cancer prevention.

Training opportunities

The student will have the opportunity to learn transferable skills, including big data analysis, data visualization, machine learning and potentially deep learning, statistics, high-throughput computing, bioinformatics, and computational genomics, including integration of large sequencing genomic, epigenomic, transcriptomic, and other data set. The interdisciplinary nature of the project will provide opportunity to also gain laboratory skills in a range of molecular biology techniques, PER-seq, and other methods. Support will be provided to develop soft skills in presenting, writing, critical thinking, experimental design, and networking within the Oxford scientific community and at conferences.

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56. Multi-cancer detection testing in clinical practice. [1,2,3,4] - Dr. Brian Nicholson

Primary Supervisor: Dr. Brian Nicholson
Second Supervisor:
Eligibility: All tracks are eligible to apply for this project.

Abstract of the project

Non-invasive MCED tests presents a new opportunity to improve early cancer detection by optimising patient selection for targeted cancer testing. Whilst MCED technologies are designed to detect a cancer signal across multiple cancer sites, their performance varies by cancer site and cancer stage. These technologies will not be used in isolation by clinicians in primary or secondary care: they will be used in people with a prior risk of cancer based on their risk factors (most importantly age), symptoms, signs, and test results, who are referred into clinical pathways for definitive testing. Care will be needed to select at-risk populations that complement the performance characteristics of the test to balance the likelihood of missed cancers and unnecessary referrals for invasive or expensive investigation. With a rapidly increasing number of MCED technologies in development, their performance characteristics are likely to improve. However, understanding the performance characteristics of MCED technologies alone will not be sufficient to guarantee the success of their implementation. Many promising innovations fail to reach clinical adoption as little attention has been given to the drivers of uptake in clinical practice. Successful clinical implementation of MCEDs in clinical practice is critically dependent upon intimate understanding of the patient, clinician and system-level factors that influence uptake. The successful candidate would join an exciting multidisciplinary programme of work investigating the accuracy, utility, and implementation of MCED testing in NHS clinical practice.

Research objectives

There is scope for the successful candidate to develop research objectives within the broad framework of the MCED focussed CRUK Oxford Cancer Centre's Early Detection theme. The Early Detection theme focusses on patient selection for MCED testing, MCED test development, and MCED test evaluation in clinical practice. The successful candidate will be supported to develop and lead research into MCED testing using methods that suit their intended career path. Examples of areas for development could be to:

- compare the performance of existing risk algorithms and clinical guidance to identify populations most at risk of cancers (combined and individually) who could be offered MCED testing by using existing health records data or by developing studies to collect new cohort data. These multi-parametric algorithms could take patterns of a patient's symptoms, signs, test results, consultation patterns, medical history and risk factors to calculate their individual risk of cancer diagnosis to be updated as MCED tests are completed.

- Utilise the Rapid Diagnostic Centre Digital Research Platform (RDC-DRP) curated to include clinical, research, and biobank data derived from the expanded Suspected CANcer (SCAN) pathway and biobank. The RDC-DRP could support fundamental and basic science researchers seeking to study early-stage disease and enhance risk factor and symptom data capture, clinical epidemiologists interested in the MCED signatures in patients with non-specific symptoms, and health services researchers hoping to use an online secure patient survey portal to collect patient data prior to and following their appointment.

- develop community-based prospective MCED cohorts and trials engaging patients across to promote diversity and inclusivity with the team who delivered the SYMPLIFY study. Together with a focus on assessing the accuracy and placement of MCED technologies within NHS clinical workflow key implementation questions could be asked using qualitative methodologies to understand the public, patient, clinician and system-level factors that influence MCED uptake and impact.

Translational potential of the project

In order for the NHS to maximise the benefit of MCEDs for patients in clinical practice research is required to understand how MCEDs complement existing diagnostic pathways, if they replace commonly used diagnostic tests, and how patients and practitioners will use them. As MCEDs develop, with improved or different analytical performance, the candidate's research findings will be required to understand where to best place MCED in the diagnostic pathway. Oxford is uniquely placed to investigate MCED technologies as the supervisory team are involved in the development of MCED technologies and NHS evaluations of MCEDs in clinical practice.

Training opportunities

In addition to the training provided by the Cancer Science DPhil programme, NDPCHS offers broad methodological expertise in applied health services research and evidence-based health care with training available to support the approach chosen by the candidate under guidance from their supervisory team. For example, the Medical Statistics group specialises in quantitative diagnostic, prognostic, monitoring, and prediction methodologies, the Medical Sociology and Health Experiences Research Group specialises in social science informed, qualitative and mixed methods implementation studies of health and illness, and the Primary Care Clinical Trials Unit delivers world class clinical trials in the community. In addition, the Oxford-led NIHR Community Healthcare MedTech and In vitro Diagnostics Co-operative (CH-MIC) works upstream and downstream of the CE-marking process to both influence the development of novel technologies and the evaluation of clinic-ready products.

57. Understanding STING regulation in cancer and the crucial role of ubiquitination in the ER – [1,3] Prof. John Christianson

Primary Supervisor: Prof. John Christianson
Second Supervisor: Assoc Prof. Eileen Parkes
Eligibility: Track 1 and 3 students are eligible to apply for this project

Abstract
Cancers interact with their surrounding environment (the tumour microenvironment) by remodelling it to contain cells promoting tumour invasion and spread, and resistance to anti-cancer therapies. Innate immune pathways, typically used to defend cells from infection by viral and bacterial pathogens, are hijacked in cancer. The mechanisms by which cancer cells modify innate immunity are currently not well understood. A key pathway is the cGAS-STING pathway – the cytoplasmic sensor cGAS recognises non-self or mislocalised DNA and activates STING (the STimulator of Interferon Genes). STING is embedded in the endoplasmic reticulum (ER) – activation of the STING-mediated interferon response requires oligomerisation and efflux from the ER (1). Fine tuning of this response is paramount, and ubiquitination of STING has emerged as an important post-translational modification capable of modulating these signalling events. Importantly, evidence is emerging of important interferon-independent effects of cGAS-STING signalling which may drive tumour progression. Establishing how ubiquitination and its conjugating machinery impact the cGAS-STING pathway is key to understanding how cancers subvert this pathway to their own ends.

This DPhil project will biochemically and functionally characterise ER-resident ubiquitination machinery that modulates STING signalling in order to delineate its regulation of the interferon response.

Research Objectives and Outcomes
Recently, our lab identified a multi-subunit complex organised around ER-resident ubiquitin ligase (E3) RNF26, whose constituents modulate signalling through STING to scale the magnitude of the interferon response (2). We are now investigating how each component of this RNF26 complex impacts STING to contribute to the response, focusing on defining protein-protein interactions, key functional domains, ubiquitin linkages, complex assembly, and its synergy (or competition) with other ubiquitin ligases. This is crucial as understanding STING regulation will identify mechanisms of resistance to immune targeting agents (immune checkpoint blockade and STING agonists) in advanced cancers.

Objective (1): Molecular dissection of ubiquitin conjugating machinery competing to modify STING in the ER. Genomic editing, gene silencing and dominant negatives will establish the individual and combinatorial contributions of ER-resident E3s (RNF26, RNF5, gp78, Hrd1) to STING properties including its; stability/degradation, ubiquitination profile, oligomerisation, trafficking, and activation of the downstream interferon response, in model cell lines. The diversity and dynamic nature of ubiquitin chain linkages modifying STING will then be explored using both mass spectrometry and sensitivity to linkage-specific deubiquitinases.

Outcome: Establishment of key ubiquitination events governing STING in the ER and consequently the magnitude of its downstream signalling cascade.

Objective (2): Defining how cofactors contribute to ER-E3 recognition and/or ubiquitination of STING. Potentially important regulatory domains of E3 complex components identified bioinformatically will be evaluated functionally using truncations and site-directed mutagenesis. This will be complemented by proximity-labeling strategies coupled with proteomics to define the spatiotemporal organisation of E3 complexes and their interaction/s with STING. STING agonists and antagonists (currently being developed for clinical applications) will be used to pharmacologically probe for changes in E3-STING interaction.

Outcome: An understanding of the how ubiquitin multifaceted regulation of STING at the ER influences response to activating treatments.

Objective (3): Preclinical validation of STING modulating factors. Identified STING regulating factors will be modified using gene editing and CRISPR-cas9 approaches using an *ex vivo* platform (i.e. culturing cells from patient samples). These samples will be used to generate organoids with/without fibroblasts and patient-matched immune cells. This near-patient system will be used to determine the effect of modulation of STING regulating factors on the tumour microenvironment. Using this platform, immunotherapeutic stimulants can be added to determine the role of STING-modulating E3s and co-factors in response to existing immunomodulating treatment will be investigated. Flow cytometry and T cell activity assays will be employed to measure the impact of novel targets on immune response in this near-patient model.

Outcome: Characterisation of targetable mechanisms of STING suppression determining response to cancer immunotherapy.

Collectively this research will develop insights into the fundamental cellular controls of immune signalling. Along with ongoing work in the lab, it will form part of our broad effort to explore and define ubiquitination events and mechanisms at the ER responsible for essential cellular homeostatic functions

Translational relevance of the project
This project will address important fundamental and clinical questions relevant to personalising immunotherapy treatment in cancer. Tailoring immune targeting approaches and understanding resistance mechanisms (such as STING repression) has potential to improving clinical responses. In this study novel STING regulating mechanisms will be characterised as potential biomarkers and/or targets for further clinical study. Moreover, this proposal uses patient samples for 3D modelling, further supporting translation of this work to the clinical setting.

Training Opportunities
There will be multiple training opportunities available during the project including; advanced cell biology and biochemistry, proteomic sample preparation and analysis, flow cytometry, and 2D and 3D cell culture modelling. There will also be opportunities to present findings at local, national and international conferences.

References

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